Resistance Screening Center Procedures Manual: A Step-by-Step Guide Used in the Operational Screening of Southern Pines for Resistance to Fusiform Rust (Revised 1988)

by

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FOREWORD

by

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Testing for Fusiform Rust Resistance

Where selecting and breeding for resistance are feasible, a plant disease often is controlled best by developing resistant varieties of plants. This rule of thumb is particularly applicable in forestry, where relatively low values per acre and long timespans between treatment and harvest usually preclude high-cost treatments. The fungicidal sprays commonly used on row crops like tomatoes or strawberries are too expensive for use in forests. That is why forest pathologists have expended so much effort to produce strains of southern pines with varying degrees of resistance to fusiform rust. This disease, caused by Cronartium quercuum (Berk.) Miyabi ex Shirai f. sp. fusiforme, is a limiting factor in the production of slash (Pinus elliottii Engelm. var. elliottii) and loblolly (P. taeda L.) pines in an area of the Southern United States extending from South Carolina to Louisiana. The new rust-resistant pines are being planted, and field tests indicate that their use will reduce disease losses by about 50 percent.

A key element in the development of rust-resistant pines was a reliable and rapid procedure for evaluating the relative resistance of host families and varieties. Field performance, of course, is the ultimate test of resistance, but field tests require 5 to 7 years, and results are uneven because disease exposure varies widely from place to place and from year to year. With the progenies of thousands of parent trees needing testing, a quick and inexpensive procedure was needed.

In the early 1970's, the Southeastern Forest Experiment Station, USDA Forest Service's forest disease research project in Athens, GA, was asked to develop the technology for large-scale testing for fusiform rust resistance with standardized numbers of spores under controlled conditions. Requirements included repeatable results closely related to field performance.

The Athens group adapted many of the procedures of cereal rust programs to the needs of the fusiform rust program. For example, vacuum-dry storage, originally developed for long-term storage of urediospores of cereal rusts, was used to retain viability of fusiform rust aeciospores for extended periods. The cyclone spore collector recommended for gathering aeciospores was developed by wheat rust researchers.

In Athens, Thomas Miller learned to concentrate and store fusiform rust basidiospores on Millipore filter pads. L. David Dwinell adapted the Coulter electronic particle counter to determine concentrations of basidiospores in suspensions. This development made it possible to prepare suspensions with standardized concentrations of spores. Fred Matthews and James Rowan developed an atomized spraying procedure that became the concentrated basidiospore spray (CBS) inoculation system. The CBS system involves seven steps:

1. Aeciospores representative of specified areas are collected.

2. These spores are screened, dried, and sealed under vacuum.

3. Juvenile leaves of northern red oak (Quercus rubra L.) seedlings are inoculated with aeciospores.

4. Abundant telial columns are produced on the undersides of oak leaves, which are suspended over acidified water at 20 °C to promote germination of teliospores and the discharge of sporidia into the water surface.

5. Sporidia are poured into a series of Millipore filters and collected on the filter pads. Spores are washed from the pads into a suspension that is diluted to the desired spore concentration after numbers present are estimated with an electronic particle counter.

6. The resulting spore suspension is applied through spray nozzles onto 6- to 8-week-old pine seedlings.

7. Responses of pine seedlings to inoculation are assessed 6 to 9 months after inoculation, depending on the tree species.

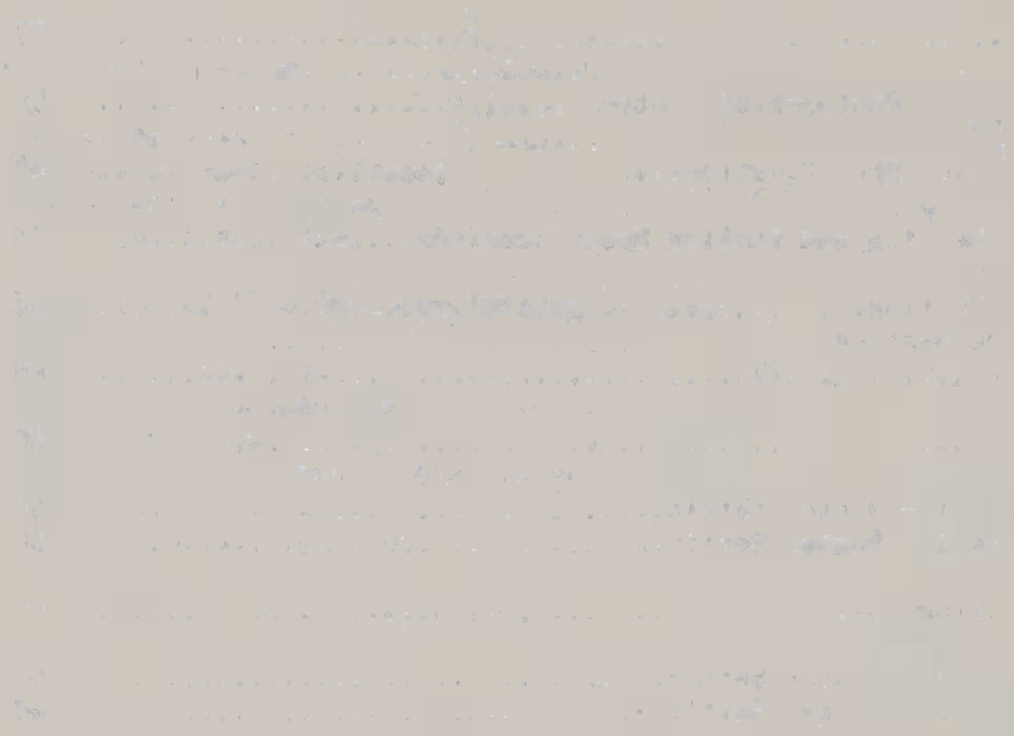
These procedures were described in a 1971 manual provided to Forest Pest Management personnel. These people were asked to convert the basic technology into a practical screening operation. The Resistance Screening Center was established at Bent Creek, NC, in 1973 with the charge of screening seedlots for all tree improvement programs concerned with selecting and breeding for fusiform rust resistance. Since 1973, many innovative improvements have been made in the basic system, and the improvement process is continuous. This manual describes procedures that were current in January 1988.

PREFACE

This manual fully describes the materials and methods used at the Resistance Screening Center to screen for fusiform rust resistance. It is designed for use by Forest Service personnel and others working at the Center.

Materials and methods used at the Center are subject to constant improvement. The procedures described here were current as of January 1, 1988. As changes occur, they will be documented and included in this manual as replacements or supplements.

Contents



Figures

AECIOSPORE COLLECTING AND PROCESSING

The fusiform rust fungus must be available to screen for resistance in pines. Fungus tissue is stored in the form of aeciospores. Spores are obtained from 13 loblolly pine and 7 slash pine collection areas. Collection areas consist of predefined locations ranging from 10 to 43 counties in size, located throughout the natural ranges of slash and loblolly pines (fig. 1). In each collection area, spores are collected from as many sites as possible. Sites must be at least 10 miles apart. Spores from at least 10 single galls at each site are mixed in equal volumes (after processing) to represent an individual collection area site.

Field Collection

Aeciospores are produced in the spring about the same time the new pine shoots reach 1 to 4 inches in length. Stands producing aeciospores are located by contacting local foresters or by windshield inspection.

1. Locate a site with galls producing aeciospores. Collect spores by one of two methods:

   a. Cyclone spore collector.--The cyclone spore collector (purchased from Iowa State University Instrument Shop) is a dual valve suction bulb which draws spores from the gall and deposits them in a small test tube. Squeeze bulb, place end of collection tube near spores, and release bulb.

   Note: If the collector tube becomes clogged with trash, insert a straightened paper clip into the end of the tube. If it is clogged with pine rosin, replace it with a clean collector. Rosin-clogged collectors may be cleaned later with alcohol.

   b. Waxed paper.--Wrap the gall in waxed paper and tap the branch beyond and behind the gall. Spores will fall onto the waxed paper. Pour spores from paper into a small test tube.

2. Plug test tube containing spores with cotton. Place in a plastic vial and cap.

3. Label vial with pine species, collection area, and site number (Ex. L-1-1).

4. Repeat steps 1 through 3 for 12 to 15 galls per site.

5. For each collection area, record the following information on an AECIOSPORE COLLECTION DATA sheet (Fig. 2).

   a. Collection area (Ex. L-1).

   b. Species (Ex. loblolly).

   c. For each collection area site, record:

      1. Weather at time of collection.

      2. Date of collection.

      3. Total number of galls collected.

FIGURE 1

RESISTANCE SCREENING CENTER

AECIOSPORE COLLECTION AREAS

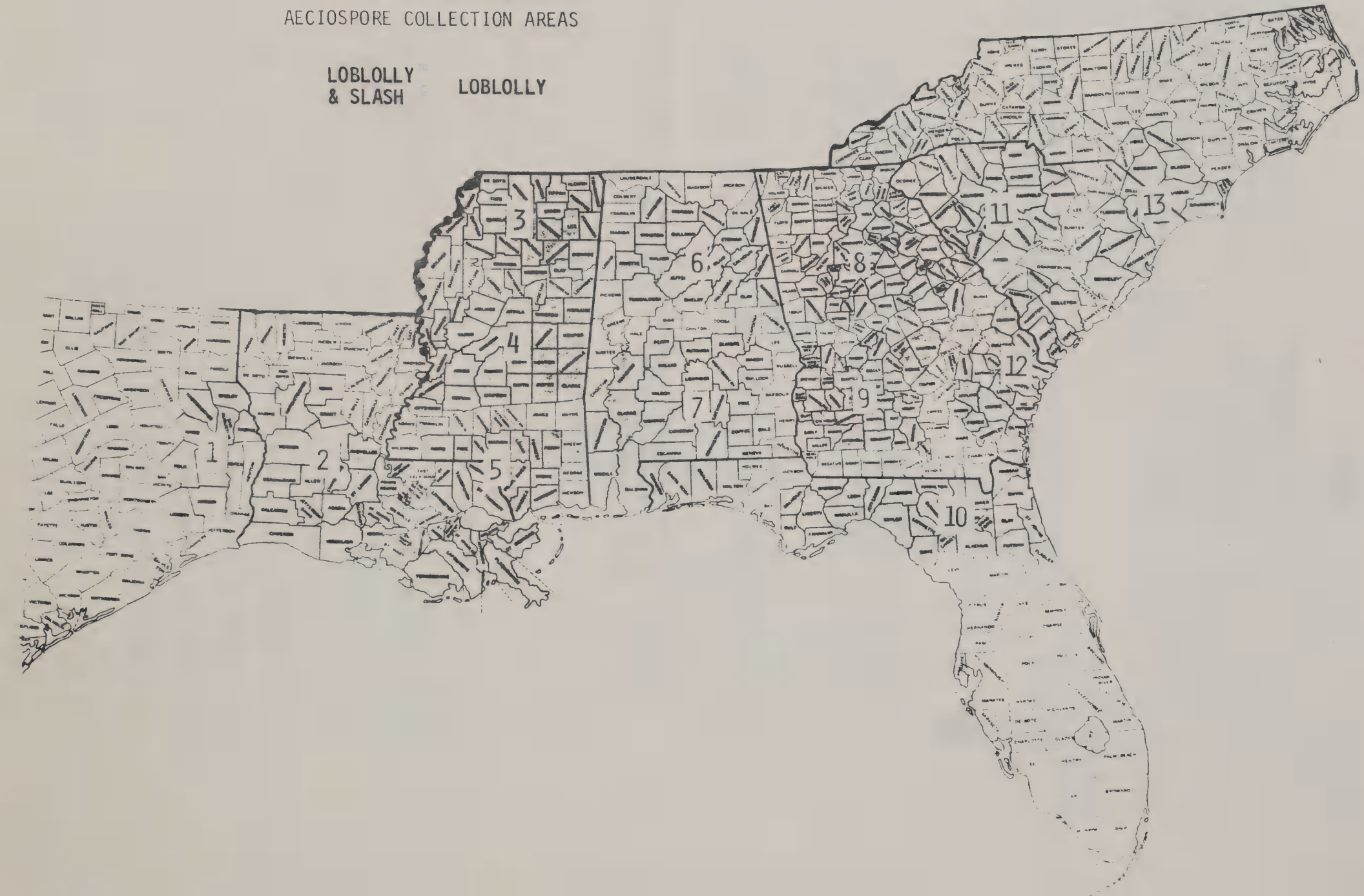


FIGURE 1 (cont'd)
RESISTANCE SCREENING CENTER

AECIOSPORE COLLECTION AREAS

Area Code	State: Counties
L-1, S-1	TX: Angelina, Hardin, Jasper, Liberty, Nacogdoches, Polk, Sabine, San Augustine, Shelby, and Tyler
L-2, S-2	LA: Acadia, Allen, Avoyelles, Beauregard, Calcasieu, Cameron, Evangeline, Grant, Iberia, Jefferson Davis, Lafayette, Natchitoches, Pointe Coupee, Rapides, St. Landry, St. Martin, Vermilion, Vernon, Winn, and West Feliciana
L-3	MS: Alcorn, Benton, Bolivar, Calhoun, Chickasaw, Clay, Coahoma, DeSoto, Grenada, Itawamba, Lafayette, Lee, Marshall, Monroe, Panola, Pontotoc, Prentiss, Quitman, Tallahatchie, Tate, Tippah, Tishomingo, Tunica, Union, Webster, and Yalobusha
L-4	MS: Attala, Claiborne, Clarke, Copiah, Hinds, Holmes, Humphreys, Issaquena, Jasper, Jefferson, Kemper, Lauderdale, Leake, Madison, Neshoba, Newton, Noxubee, Rankin, Scott, Sharkey, Simpson, Smith, Warren, Winston, and Yazoo
L-5, S-5	MS: Amite, Forrest, Hancock, Harrison, Lamar, Marion, Pearl River, Perry, Pike, Stone, and Walthall
	LA: Ascension, Jefferson, Lafourche, Livingston, Orleans, St. Charles, St. Helena, St. James, St. John the Baptist, St. Tammany, Tangipahoa, Terrebonne, and Washington
L-6	AL: Blount, Calhoun, Cherokee, Clay, Cleburne, Cullman, Etowah, Fayette, Jefferson, Lawrence, Marshall, Morgan, Randolph, Shelby, St. Clair, Talladega, Tuscaloosa, Walker, and Winston
L-7, S-7	AL: Autauga, Baldwin, Bullock, Butler, Clarke, Coffee, Conecuh, Covington, Crenshaw, Dale, Dallas, Elmore, Escambia, Geneva, Lowndes, Macon, Marengo, Mobile, Monroe, Montgomery, Perry, Pike, Washington, and Wilcox
	FL: Escambia, Okaloosa, Santa Rosa, and Walton
L-8	GA: Baldwin, Banks, Barrow, Bartow, Butts, Cherokee, Clarke, Clayton, Cobb, Coweta, De Kalb, Douglas, Fayette, Forsyth, Fulton, Glascock, Greene, Gwinnett, Hall, Hancock, Henry, Jackson, Jasper, Jones, Lamar, Madison, McDuffie, Meriwether, Monroe, Morgan, Newton, Oglethorpe, Oconee, Paulding, Pike, Putnam, Rockdale, Spalding, Taliaferro, Upson, Walton, Warren, Washington, and Wilkes

L-9, S-9	GA:	Baker, Ben Hill, Berrien, Bleckley, Calhoun, Chattahoochee, Clay, Colquitt, Cook, Crisp, Dodge, Dooly, Dougherty, Early, Houston, Irwin, Lanier, Laurens, Lee, Macon, Marion, Miller, Mitchell, Peach, Pulaski, Randolph, Quitman, Schley, Stewart, Sumter, Telfair, Terrell, Tift, Turner, Webster, Wilcox, and Worth
L-10, S-10	FL:	Alachua, Baker, Bradford, Calhoun, Clay, Columbia, Dixie, Duval, Flagler, Franklin, Gadsden, Gilchrist, Gulf, Hamilton, Jefferson, Lafayette, Leon, Liberty, Madison, Nassau, Putnam, St. Johns, Suwannee, Taylor, Union, and Wakulla
L-11	SC:	Abbeville, Aiken, Anderson, Chester, Edgefield, Fairfield, Greenwood, Kershaw, Lancaster, Laurens, Lexington, McCormick, Newberry, Richland, Saluda, Union, and York
L-12, S-12	GA:	Appling, Bacon, Brantley, Bryan, Bulloch, Chatham, Effingham, Evans, Glynn, Liberty, Long, McIntosh, Pierce, Screven, Tattnall, Toombs, and Wayne
	SC:	Allendale, Bamberg, Beaufort, Colleton, Hampton, and Jasper
L-13	SC:	Berkeley, Clarendon, Dillon, Florence, Georgetown, Horry, Marion, and Williamsburg
	NC:	Bladen, Brunswick, Columbus, Cumberland, Hoke, New Hanover, and Robeson

FIGURE 2

AECIOSPORE COLLECTION DATA

COLLECTION AREA _____

SPECIES _____

_____

COLLECTION I DATE _____

WEATHER _____ TOTAL # GALLS _____

SITE LOCATION _____

_____

_____

_____

COLLECTION II DATE _____

WEATHER _____ TOTAL # GALLS _____

SITE LOCATION _____

_____

_____

_____

COLLECTION III DATE _____

WEATHER _____ TOTAL # GALLS _____

SITE LOCATION _____

_____

_____

_____

REMARKS:

4. Location of site. Be as specific as possible. Give road map directions and other pertinent information.

6. Place all vials from a single site in a plastic bag and seal. Mark bag with collection area and site identification (Ex. L-1-1). Store spores in ice chest. Avoid getting spores wet.

7. Use a clean spore collector at each site. Sterilize all spore collectors at the end of each day. To sterilize:

   a. Disassemble spore collector and rinse in 70 percent alcohol.

   b. Rinse thoroughly in water.

   c. Dry thoroughly (usually overnight).

   d. Reassemble.

   Note: Several galls may be collected with the same spore collector at the same site. Pump collector several times between gall collecting without test tube attached.

8. Allow enough time at the end of each collection day to individually screen spores from each gall through a 50-mesh sieve screen to remove insects and trash.

   If spores are damp, they should be air dried in an air-conditioned or heated room for 1 or 2 hours before screening.

   Sterilize sieve screens between each site. To sterilize:

   a. Rinse screen in 70 percent alcohol.

   b. Rinse thoroughly in water.

   c. Dry thoroughly. (A hair dryer can be used to speed the process. Allow screen to cool before reusing.)

9. Once they are screened, return spores to original test tube, replug with dry cotton if original plug is damp, place in plastic vial with cap, doublecheck label, and store in ice chest during transport to laboratory. Place in a 5 ^{o}C incubator upon return to laboratory.

Laboratory Processing

Germination Check

1. Place three small test tubes in a holder.

   Fill one tube with 70 percent alcohol, one tube with distilled water, and partially fill one tube (1/4 to 1/2 full) with distilled water.

2. Remove vials of spores from one collection area from incubator. Number vials, beginning with 1.

3. Label petri plates of 2 percent Nobles agar with collection area, site, and vial number (Ex. L-1-1-1).

4. Place a small sample of spores from a single vial into the partially filled tube of distilled water. Place thumb over end of tube and shake vigorously, or stopper and agitate using tube shaker.

5. Spray petri plate with spore suspension using a Spra-Tool Power Pak hand-held sprayer. Hold plate 4 or 5 inches from spray nozzle. Shake excess liquid from plate and cover.

6. To clean hand-held sprayer, dip sprayer tube in alcohol and spray a couple of seconds. Shake excess alcohol from spray tube and immerse in the full test tube of distilled water. Spray entire contents of this tube.

7. Dispose of tube with spore suspension and replace with clean tube partially filled with fresh distilled water. Replenish the alcohol and distilled water. Replace the alcohol and fill distilled water test tubes after every third use.

8. Repeat steps 2 through 7 for all vials of spores.

9. Incubate all plates at 20 ^{o}C for 24 hours.

10. Using a microscope at 30x, tally the number of spores germinated out of 100 total spores.

    Record germination as a percent on the AECIOSPORE PROCESSING DATA sheet (fig. 3). Fill in collection area and date of evaluation, as well as other required information.

11. Repeat step 10 for all vials of each collection area, by collection area, site, and vial number.

Combining Spores to Represent Collection Area Sites (Mixing Spores)

1. Gather all vials from one site in a collection area. Discard those with 15 percent germination or less, if there are at least 10 vials per site with better germination.

2. Choose 10 vials from each site with the most spores.

3. Determine the lowest weight of spores in these 10 vials. Remove this weight of spores from each vial. Record weight on the AECIOSPORE PROCESSING DATA sheet.

4. Combine and screen spores from the 10 galls through a 100-mesh sieve screen. Mix spores thoroughly.

5. Remove a small amount of mixed spores and suspend in distilled water. Spray a 2 percent Nobles agar plate with the suspension. After 24 hours incubation at 20 ^{o}C, evaluate germination of 100 spores and record as percent germination of mix before desiccation on the AECIOSPORE PROCESSING DATA sheet.

6. Repeat steps 1 through 5 for each site from each collection area.

FIGURE 3
AECIOSPORE PROCESSING DATA

Collection Area_____ Date_____

Vial #	% germination			used✓
Collection	I	II	III	III
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				

% germination of mix before desiccation_____

% germination of mix after desiccation_____

Volume of each used in mix_____

Remarks:

First Desiccation

1. Place spores of one site in open 100- by 15-mm petri dishes to a depth of 3 to 5 mm. Label bottom of dish with collection area, site, and date.

2. Prepare desiccator by filling bottom with anhydrous calcium chloride. Cover with round, porcelain plate.

3. Stack dishes of spores in desiccator. Grease lid of desiccator with stopcock grease and cover.

4. Attach vacuum pump tube and evacuate desiccator to 25 to 27 inches of mercury. Close valve of desiccator.

   Label lid of desiccator with collection area(s), site(s), and date.

5. Refrigerate for 5 to 7 days. Longer drying periods are not harmful.

Second Desiccation

1. Remove desiccator from refrigerator and allow to warm to room temperature.

2. Carefully and slowly release vacuum.

3. By individual site, remove a small amount of spores and rehydrate by placing spores in an open 35- by 10-mm petri dish.

   a. Place this dish in a 100- by 15-mm petri dish lined with moist filter paper. Cover and label lid with collection area, site, and date.

   b. Refrigerate for 24 hours at 5 ^{o}C.

   c. Spray a 2-percent Nobles agar plate with spores and incubate 24 hours at 20 ^{o}C, and evaluate germination of 100 spores. Record as percent germination of mix (by site) after desiccation on the AECIOSPORE PROCESSING DATA sheet.

4. Turn on the 72-port vertis manifold (part of a freeze-drying unit) attached to a Welch duo-seal vacuum pump (the "cow") and check oil level on side of pump. Be sure all ports are closed before turning pump on.

5. Use a modified thistle tube for a funnel to deposit approximately 0.1 g of spores into a 1-mL "tear-drying bulb" (ampoule). Tapping funnel gently will help spores fall into ampoule.

6. Plug ampoules with cotton. Plugs that are too loose allow spores to be sucked into vacuum system. Plugs that are too tight increase the time required to draw a vacuum.

7. Lightly grease necks of ampoules with stopcock grease.

8. Place all ampoules on "cow." Keep ampoules of same collection area site together and label accordingly.

9. Open each port separately by placing thumb over end of port and slowly turning the value 180 degrees.

Open 3 or 4 ports, then stop and allow vacuum to come down to 50 μ before continuing.

10. After all ports in use are opened, monitor vacuum with a McLeod gauge every few minutes until a minimum of 50 μ of mercury is reached. Maintain 50 μ or less for 1 hour.

11. Flame seal each ampoule with a natural gas-air torch.

    a. Connect one plastic tube on torch to a natural gas supply and the other plastic tube to an air supply.

    b. Adjust the gas-air mixture so that a narrow, blue flame is obtained.

    c. Seal the ampoule approximately midway up its neck by applying flame to sealing point and allowing the glass to evenly heat. As glass begins to melt, twist bulb of ampoule to create closure at seal point. Gently pull down and out while twisting. Seal as quickly as possible to avoid overheating of ampoule, which will kill spores. Care should be exercised to avoid damage to ports. Special care should be taken to avoid pulling ampoule out of port while sealing. If an unsealed ampoule leaves the port, close port and allow vacuum to return to 50 μ and maintain for 15 minutes before continuing.

       As sealed ampoules are pulled away, a fine thread of glass will be left. This should be heated with the torch at the sealing point and pushed backward as glass heats up. This eliminates the possibility of breaking these tips and losing the vacuum.

12. Lay sealed ampoule on countertop to cool. Avoid tilting spores into neck while hot.

13. When cool, place all ampoules of one collection area site in a cloth bag and attach a paper label. Mark bag with inoculum code for source area, site, and year (Ex. L-5-1-86).

14. Store bags in a 5 ^{o}C incubator. Add information to inventory notebook in lab under appropriate area code.

15. Place AECIOSPORE COLLECTION DATA sheet, AECIOSPORE PROCESSING DATA sheet, and any other information pertaining to the collection in the appropriate office file.

ACORN COLLECTION AND STORAGE

Northern red oak seedlings must be available in order to perform a screening test. Acorns are kept on hand at all times. Northern red oak acorns are collected from any location with an abundance of acorns. Collection areas often differ from year to year because of the cyclical nature of acorn production.

Acorns are heat-treated, dried, and placed in plastic bags for cold storage. Heat-treating acorns kills acorn weevil larvae.

Acorn Collection

Collect acorns from northern red oak trees and transport to the Resistance Screening Center. Record date and location of collection. Place acorns in cooler in the soil building until they are processed for storage. To avoid acorn dehydration and damage from acorn weevil larvae, acorns should be processed within 5 days after collection.

Acorn Processing

1. Allow acorns to warm up to room temperature, if they have been stored in a cooler.

2. Fill controlled-temperature water bath approximately three-fourths full with warm water (120 ^{o}F). Mount a thermometer on one side of the water bath. Place a fitted wire basket into the water bath.

3. Pour acorns into basket in water bath so that they are completely covered with water. Remove and discard acorns that float. These acorns are generally unsound and usually will not germinate. Stir acorns in water bath several times and continue to remove acorns that float.

4. Maintain water temperature at 120 ^{o}F ($\pm$ 1^{o}) for 40 minutes. Because water temperature drops when acorns are added, do not start timing until water returns to proper temperature.

5. Lift basket out of water bath and allow water to drain. Spread acorns one layer thick on a wire drying rack. Allow to air-dry overnight.

6. Place acorns in plastic ziploc bags (6- by 6-inch, which hold 200-300 acorns, are preferred). Fill bags approximately one-half full. Add 1/2 teaspoonful Captan powder (fungicide) to each bag and shake to spread. Remove excess air and seal bag. Number bags consecutively, beginning with 1 plus the collection year (Ex. 1-86).

7. Tabulate an acorn collection sheet by recording bag numbers, year, collection area, collection date, and date treated in water bath.

   Example:

Bag Numbers	Year	Area	Date Collected	Date Treated
1 thru 20	1986	Bent Creek	10/10/86	10/11/86

Acorn Storage

Place bagged acorns in cooler in soil building. Mark shelves with collection year. Cooler is maintained between 33 and 38 ^{o}F. Acorns can be stored approximately 3 years without total loss of viability.

Acorns should be checked at least every 6 months for signs of mold. Bags with heavy mold should be discarded.

TEST INITIATION AND SCHEDULING

An operational screening test is initiated when seedlots are received from a client. A completed RESISTANCE SCREENING REQUEST SHEET should accompany all seed. Client name, phone number, pine species, date seed sent, whether or not client wants leftover trees, invoice address, first and second copy of results addresses, seedlot names, and inoculum source areas are designated on this form (fig. 4).

The operational test design involves two replications in time; that is, two inoculations on consecutive days (runs). On each day, three 20-tree trays from each seedlot are inoculated. Thus, each seedlot is represented by 120 trees. The timing of test operations is as follows.

A. Pine Schedule

1. Stratification (S) - loblolly only

2. Pine plant (PP) - 8 weeks from S

3. Pine transplant final (PTF) - 3 weeks from PP

4. Pine inoculation (PI) - 5 weeks from PTF

5. Fertilization (PF) first - 3 weeks from PI

6. Programmed pine fertilize (PPF) - 21 days after PF. On this date, the test can be included in the planned fertilizing schedule of every 6 weeks.

7. Reading (R) - 25 weeks after PI for slash and 29 weeks after PI for loblolly

   Note: Readings may be initiated 1 week prior to the scheduled date and completed as late as 1 week after the scheduled date.

B. Oak Schedule

1. Oak plant (OP) - 2 days after PP

2. Rehydrate (RH) - minimum of 24 hours prior to OI

3. Oak inoculate (OI) - 4 weeks after PP. The actual oak inoculation date may vary from this by 2 to 7 days, depending on the rate of development of the oak leaves.

4. Harvest telia (HT) - 3 weeks after OI. Telia may be harvested as early as 2-1/2 weeks or as late as 4 weeks after the actual oak inoculation date.

5. Mix dilution (MD) - 1 day before PI of each run

FIGURE 4

RESISTANCE SCREENING REQUEST SHEET

RESISTANCE SCREENING CENTER
USDA FOREST SERVICE, RT. 3, BOX 1249A, ASHEVILLE, NC 28806
(Comm. 704-667-5089, 704-665-2186)

A test consists of not more than 50 seedlots to be inoculated with spores from one inoculum source area (or a mix of inoculum areas). One request sheet should be submitted for each test. Two hundred and fifty good-quality seed are requested for each seedlot. Four hundred good seed are requested for seedlots with poor germination. Seedlots containing at least 150 good-quality seed can be screened and should be labeled as such.

Client _____ Phone # _____ Species _____

Date seed sent _____ Do you want the leftover trees from the seedlots? Yes No (circle one) (IF, YES, PLEASE SEE BACK OF FORM.) Research has shown that noninfected seedlings from the more resistant seedlots are genetically superior to the original collection. This is not necessarily true for more susceptible seedlots.

Mark inoculum source area(s) on map on reverse side of form.

Invoice to:

_____
_____
_____
_____

First copy of results to:

_____
_____
_____
_____

Second copy of results to:

_____
_____
_____
_____

Seedlot Names	Seedlot Names	Seedlot Names
1	18	35
2	19	36
3	20	37
4	21	38
5	22	39
6	23	40
7	24	41
8	25	42
9	26	43
10	27	44
11	28	45
12	29	46
13	30	47
14	31	48
15	32	49
16	33	50
17	34	

Evaluations at the screening center have shown that seedlings can be shipped by bus with a great deal of success in the spring and fall (if the time in transit is held to 2-3 days). Seedlings shipped in the winter and summer have an increased risk of temperature damage. The degree of damage will depend on the time in transport and temperature exposure.

Because of the cost of separating and packing, there will be an additional charge of $3.00 pe seedlot screened. Also, seedlings will be sent c.o.d. by bus.

a. Make a circle on the map. (All stands of the selected host within the circle will be mixed.)

b. Make a dot on the map. (The 3 closest stands will be mixed.)

Examining Client's Seed

1. Examine seed. If seed are found to be in good condition, notify client that seedlots were received in good condition and the date received. If seedlots are damaged, notify client at once.

2. Match seedlot names on RESISTANCE SCREENING REQUEST SHEET with those on containers holding seed. If a discrepancy is found, notify client at once.

Scheduling Test

1. Assign next available test number. Operational test numbers have 5 digits.

   1st digit = 1 = operational design
   2d and 3d digits = number of test, beginning at 01.
   4th and 5th digits = year seedlots will be inoculated.

   Example: 110-86 = operational test 10 - inoculated in 1986.

2. Using magnetic scheduling board, determine earliest possible inoculation date based on available greenhouse space.

3. Determine the Julian calendar date of inoculation for Run A. This is Tuesday of the week selected for inoculation.

   Example: Inoculation week is August 25. Tuesday of that week is August 26. August 26 = 238 Julian date.

4. Print out a sample work schedule and magnetic board information sheet by running the HELLO WORK SCHEDULE program on the Apple IIE.

5. Locate HELLO WORK SCHEDULE disk in file box and insert in disk drive I of Apple IIE. Turn on computer. Program is automatically loaded.

6. Answer program questions accordingly.

   Example:

Computer Response	User Response (followed with RETURN)
Please remember to set forms to top of page.	
Do you want magnetic board info?	Yes/No
Enter the test number.	101-86
Enter the species.	Loblolly/slash
Enter the client's name or test title.	Sample run
Is this an operational test?	Yes
Enter the number of seedlots.	25
Enter the inoculum source.	L-#/S-#
Enter the number of acorns to plant.	175*
Enter the number of oaks to inoculate.	25*
Enter Julian date of Run A inoculation.	238
Enter year of inoculation.	86

* As determined in Oak Germination and Transplanting section.

7. Computer will then calculate work schedule dates and print out a complete work schedule and magnetic board information sheet, if requested.

8. If "Is this an operational test?" response is no, additional questions will be asked by the computer.

   Example:

Computer Response	User Response
Is this an operational test?	No
Enter number of seedlots.	25
Enter number of trays per seedlot per run.	4
Enter number of runs.	3
Enter number of checks.	3
Enter checks.	(names of seedlots)

9. Check additional information on magnetic board.

   Location of test before and after inoculation.

   Discard or ship seedlings after reading complete.

Prepare Forms

Work schedule form (fig. 5). This form is done on the Apple IIE computer, as described above. The following information is required.

1. Test number
2. Species
3. Client
4. Test design - operational - yes
5. Inoculum source
6. Number of oaks to plant
7. Number of oaks to inoculate
8. Julian date of Run A pine inoculation
9. Year of inoculation

Test tray tally (fig. 6). The following information is required.

1. Species
2. Test number
3. Client
4. Stratification date (loblolly only)
5. Planting date
6. Pine transplant final date
7. Pine inoculation dates
8. Seedlot designations

Inoculation sheet (fig. 7). The following information is required.

1. Species
2. Test number
3. Inoculum source

FIGURE 5

WORK_SCHECULE

SPECIES _____ TEST NO. _____

CLIENT _____

TEST_DESIGN : Op. ____ No. Seedlots _____ No. Checks ____ Total _____
No. Trays/Seedlot/Run _____ No. Trays/Run _____ No. Runs _____

CHECKS _____

READINGS _____

SPECIAL INSTRUCTIONS : _____

_____

_____

INOCULUM SOURCE _____

NO. ACORNS TO PLANT _____ NO. OAKS TO INOCULATE _____

BASIDIOSPORE DILUTION DENSITY _____ SP/ML

SPECIAL DILUTION INSTRUCTIONS : _____

_____

_____

(S) STRATIFY _____

(PP) PLANT _____ (OP) PLANT ACORNS _____

(PTF) TRANS. FINAL _____ (RH) REHYDRATE _____

(PI) INOCULATE _____ (OI) OAK INOC. _____

(PF1) FERTILIZE _____ (HT) HARVEST TELIA _____

(PPF) PROG. FERTILIZE _____ (MD) MIX DILUTION _____

(R) READING _____

REMARKS : _____

_____

_____

_____

FIGURE 6
TEST TRAY TALLY

Species _____ Client _____ Test No. _____

(S) _____ (PP) _____ (PTF) _____ (PI) _____

Dataset Name _____ (R)_____ (R) completed Run A _____

Reader _____ Run B _____

Seedlot Designation	G.H. Code	Run A									Run B								
		Tray 1			Tray 2			Tray 3			Tray 1			Tray 2			Tray 3		
		TR.	IN.	R.	TR.	IN.	R.	TR.	IN.	R.	TR.	IN.	R.	TR.	IN.	R.	TR.	IN.	R.
	1																		
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		
	8																		
	9																		
	10																		
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	22																		
	23																		
	24																		
	25																		
	26																		
	27																		
	28																		
	29																		
	30																		

TR = Transplant IN = Inoculated R = Read

10/19/84

FIGURE 7
INOCULATION SHEET

Species _____ Test No. _____

Run _____

Inoculum Source _____

Inoculation Date _____ Time: Begin _____ End _____

Temp: Begin _____ End _____

Calibration:

Position 1: Begin _____ End _____ Nozzle No. _____

Position 2: Begin _____ End _____ Nozzle No. _____

Pressure: Begin _____ End _____

Weather Conditions: _____

Inoculation Tray Order: _____

_____

Chamber Removal: Date _____ Time _____ Temp _____

Transport to Plastic House: Date _____ Time _____ Outside Temp _____

Run _____

Inoculum Source _____

Inoculation Date _____ Time: Begin _____ End _____

Temp: Begin _____ End _____

Calibration:

Position 1: Begin _____ End _____ Nozzle No. _____

Position 2: Begin _____ End _____ Nozzle No. _____

Pressure: Begin _____ End _____

Weather Conditions: _____

Inoculation Tray Order: _____

_____

Chamber Removal: Date _____ Time _____ Temp _____

Transport to Plastic House: Date _____ Time _____ Outside Temp _____

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Greenhouse record (fig. 8). The following information is required.

1. Species
2. Test number

Prepare Files

Place copies of forms in the following locations. (Number of copies are indicated in parentheses.)

Greenhouse folder (accompanies seed and seedlings throughout screening test).

Work schedule (1)
Magnetic board information (1)
Test tray tally (1)
Inoculation sheet (1)
Greenhouse record (1)

Test folder (remains in office filing cabinet).

Test checklist
Letter accompanying seed
Work schedule (1)
Magnetic board information
Notification of seed received
Request for screening
Test tray tally (1)
Assessment forms
Letter informing client of schedule
(Dilution forms and results reports are placed in this folder as they are completed.)

Lab notebook - WORK SCHEDULE.

Working notebook in office - WORK SCHEDULE, TEST TRAY TALLY sheet.

Update Calendar

Transfer the following dates onto the WORK CALENDAR (scheduled day-to-day activities located in office).

Pine schedule

1. Pine stratification (S)
2. Pine plant (PP)
3. Pine transplant final (PTF)
4. Pine inoculation (PI)
5. Pine fertilize (PF)
6. Programmed pine fertilize (PPF)
7. Reading (R)

Figure 8 - GREENHOUSE RECORD

Bay Location _____ Test No. _____

Species _____ Total No. Trays Inoculated: Run A _____ Run B _____

Run C _____ Run D _____

	Date	Type & Concentration	Run	#Trays	Initials
Fertilizer Applications:					
Insecticide Applications:					
Seedling Condition:					

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Oak schedule

1. Oak plant (OP)
2. Rehydrate (RH)
3. Oak inoculate (OI)
4. Harvest telia (HT)
5. Mix dilution (MD)

Inform client by letter of inoculation date and month in which he can expect results (expect results within 2 months after scheduled reading date).

OAK GERMINATION AND TRANSPLANTING

Northern red oak acorns are stratified a minimum of 90 days. Acorns require 5 to 14 days to germinate.

Germinating Acorns

1. Determine test number and number of acorns to be planted. This information is found on the master calendar in main office and on individual test work schedules.

   Note: Number of acorns to plant for a test is determined by the number of seedlots in a test (Ex. 10-20 seedlots = 120-130 acorns; 21-30 seedlots = 130-140 acorns; 31-50 seedlots = 140-150 acorns). Excess planting is done to allow for uneven germination, poor germination, and loss of some plants due to poor root development or poor-quality oaks. It assures that sufficient oaks required for inoculation are ready at the scheduled time.

2. Use oldest acorns first.

3. Inspect several bags and select acorns that have sprouted.

4. Place acorns in a bucket of water. Discard all acorns that float, are molded, or have dark spots or worm holes.

   Note: If sprouted acorn roots are 1 to 1-1/2 inches in length, place in clean plastic bag, label with test number, and return to cooler. At first transplanting of germinated acorns for that test, follow steps 2 and 3 of Transplanting Germinated Acorns. Place these transplants with others for that test.

5. Fill 13-1/2- by 4-1/2- by 5-1/2-inch sterilized plastic trays to within 1 inch of the top with clean vermiculite. Plant 32 to 36 acorns per tray in 8 to 9 rows of 4 acorns across. Place acorns on their sides on top of vermiculite, with root tip pointed slightly downward. Sprinkle a light covering of vermiculite over acorns. Do not completely cover acorns. Place a plastic tag in each tray marked with the date planted and test number.

6. Gently water trays, being careful not to wash away the covering of vermiculite or disturb acorns.

7. Place trays in germination bed. (Germination bed provides bottom heat and automatic watering twice a day.)

Transplanting Germinated Acorns

1. After 5 to 7 days in germination bed, examine acorns for sprouting. Gently pull on each acorn to remove from tray. Vermiculite may need to be loosened by carefully inserting fingers down and under roots and gently lifting upward. Transplant acorns with roots 1-1/2 inches long or longer. Avoid using acorns with thin, weak root systems or roots with mold or discoloration. After examining all acorns for sprouts, return acorns which are not ready for transplanting to germination tray. Combine these acorns to reduce number of trays and return to germination bed for another 5 to 7 days.

2. Transplant germinated acorns into clean, 4-inch diameter plastic pots.

   Tilt pot to one side and partially fill with moistened artificial planting medium. (Terralite Metro Mix #300 is currently being used.)

   Place acorn in pot with root system down, and gently fill other side of pot. Sit pot upright. Check to see that the acorn is at the soil level. Press medium around root, taking care not to break or disturb the root. Additional medium may need to be added. Packed medium should come to within 1 to 1/2 inch of top of the pot.

3. Place transplanted acorns in Bay 1 of glass greenhouse.

   Water carefully.

4. For acorns that were returned to germination bed after first examination, repeat steps 1 through 3 at appropriate time. Discard vermiculite and ungerminated acorns after second transplanting.

5. Thoroughly wash acorn germination trays, soak in 10 percent chlorox solution for 10 minutes, rinse in tapwater, and allow to air dry before reusing.

OAK INOCULATION

Young oak seedlings are inoculated with designated aeciospore sources.

Rehydrating Aeciospores

1. Remove ampoule(s) of designated aeciospores from 5 ^{o}C incubator in the lab. The inoculum source to be used in a test is recorded on each test WORK SCHEDULE and on the WORK CALENDAR in the office. Enter the date, number of ampoules removed, and test number in the Aeciospore Inventory Book, which is located in the rust lab.

2. Use a glass tube cutter to open ampoule(s). Empty contents of one ampoule into a 25-mm petri dish and leave uncovered.

3. Line a 60-mm petri dish with two filter papers and moisten with distilled water.

4. Place smaller petri dish on top of moistened filter paper inside larger dish. Place lid on larger petri dish.

5. Mark lid with test number, inoculum source (include source year), and date rehydrated. Store in 5 °C incubator a minimum of 24 hours and a maximum of 10 days prior to inoculation.

6. To mix aeciospore sources, repeat steps 1 through 5 for each source. Keep separate until preparation of spore suspension.

Preparing Spore Suspension and Inoculating Oaks

1. To mix aeciospore sources, weigh out equal volumes of each source, place in a clean petri dish, and mix thoroughly. Weigh out enough spores at each source so that the total volume of the aeciospore mix is at least 0.3 g.

2. Place 0.12 g of rehydrated (mixed) aeciospores into a plastic, graduated bottle which can be used with Power-Pak hand-held sprayer. Add 100 mL distilled water. Screw lid on plastic bottle and shake vigorously for several minutes to suspend spores.

   Place 0.24 g of rehydrated aeciospores in 200 mL distilled water, if 25 or more oaks are to be inoculated. Shake vigorously for several minutes to suspend spores.

3. Remove a 2-percent Nobles agar petri dish from 5 °C incubator. Mark lid with test number, inoculum source, and date of inoculation.

4. Transport spore suspension and agar plate to greenhouse.

5. Select oak seedlings for inoculation. Select healthy seedlings with large, yet tender leaves. A minimum of 20 oaks are inoculated for operational tests. The number of inoculated oaks required is recorded on the test WORK SCHEDULE and on the WORK CALENDAR.

   The number of oaks to inoculate is determined by the number of seedlots in a test.

   10 seedlots or less = 20 inoculated oaks
   11-20 seedlots = 20-25 oaks
   21-30 seedlots = 25-30 oaks
   31-40 seedlots = 35-40 oaks
   41-50+ seedlots = 40 oaks

   If all required oaks are not available at the same time, then inoculate as many as possible and continue inoculating for 3 to 4 days until total number of oaks required is reached. The same spore suspension can be used for several inoculations, if it is refrigerated when not in use.

6. Label each oak with a plastic pot label. Mark each pot label with test number. Every fifth label should also show inoculum source and date of inoculation.

7. Change the humidifier electric plug on the inoculation chamber from the timed outlet to the untimed outlet (number 16).

8. Attach bottle of inoculum to a Power-Pak hand-held sprayer.

9. Spray spore suspension on undersides of oak leaves while leaves are supported against a wire frame. Spray in a slow, circular pattern to thoroughly cover leaf undersides with small mist-size droplets.

10. Place each oak in the inoculation chamber immediately after inoculating. Make sure leaves of adjacent oaks do not touch or overlap.

11. Spray the plate of Nobles agar with spore suspension. Place plate in inoculation chamber with the lid ajar. Close chamber door. Turn off chamber light.

12. After 5 minutes, return humidifier plug to the timed outlet.

13. Place leftover inoculum in refrigerator in headhouse. If not used within 4 to 5 days, discard. If more is required, mix fresh inoculum.

Removing Oaks from Chamber

1. Remove oaks from inoculation chamber after 24 hours. Place on greenhouse carts and store in headhouse out of direct sunlight for 24 hours.

2. Remove Nobles agar plate from inoculation chamber and transport to lab. Using Leitz microscope at 30x, observe 100 spores and, using a mechanical counter, count the number that have germinated and the number that have not germinated. Record the number germinated as percent germination on the chart beside the microscope and in the Aeciospore Inventory Notebook.

3. After 24 hours in headhouse, place inoculated oaks in first bay of glass greenhouse on a bench with drip-tube watering system. Place a drip-tube watering device in each pot. Leaves of adjacent oaks should not touch or overlap. All oaks for a test should be grouped together.

4. Water often enough to keep soil damp but not soggy.

5. To water, turn on faucet attached to drip-tube water line. Allow to run for several minutes. Check each drip tube to be sure water is reaching soil.

BASIODIOSPORE PRODUCTION AND COLLECTION

Basidiospores can be induced to form on telia as early as 15 days after inoculation, reaching maximum production at 21 days. Good basidiospore production continues for at least 1 week. Once collected, basidiospores can be stored for several weeks.

The process requires work on each of 2 days.

Day 1: Induce Basidiospore Formation (Harvest Telia)

1. Cut leaves from oaks scheduled for harvesting (HT on work schedule). Place one-half of the harvested leaves in one plastic bag and the remaining half in a second plastic bag. Place a label from an oak pot in each bag. Be sure the label includes test number and inoculum source. One bag will be used for Run A and the other bag for Run B.

2. Transport bags of leaves to rust lab. Protect bags under coat jacket or in an ice chest during extreme outside temperatures.

3. Dump leaves for Run A on work counter. Set aside leaves with poor telial coverage.

4. Arrange twenty 125-mm filter paper disks on counter.

5. Select leaves with the best telial coverage. Flick the top side of each leaf with the forefinger to knock off urediospores and dirt particles. Arrange leaves on all 20 disks (usually 2-3 leaves).

6. Staple leaves to disks with the upper surface of the leaf against the filter paper. Use as few staples as possible (2 per leaf) and avoid curling filter paper. Cover as much of the disk as possible without overlapping leaves or extending beyond edge of disk.

7. Moisten lids of 150-mm diameter plastic disposable petri dishes with distilled water from a squirt bottle. Place a disk with stapled leaves paper side down in each lid. Water absorbed by the filter paper causes the paper to adhere to the lid. Pour excess water out of each lid.

   Be sure disks are as flat as possible against lids. Press gently on each staple after paper has absorbed water from lid.

8. Place 45 to 50 mL of acidified distilled water (pH 2) from a 20-L container on shelf over counter into the bottom of a petri dish.

   Acidified distilled water (pH 2) is stored in a labeled 20-L container. pH 2 is obtained by filling the container (when empty to discharge valve) with distilled water and adding concentrated liquid hydrochloric acid until a constant reading of pH 2 is obtained on the pH meter. Approximately 25 to 75 mL of acid are required. Small amounts of acid should be added each time and mixed thoroughly between readings. If too much acid is added and pH reading is too acidic (less than 2), then water must be drawn out and plain distilled water added. Continue making adjustments until pH 2 reading is obtained. Caution should be used when working with acid.

9. Place lid with filter paper onto bottom part of dish. Leaves should be facing the bottom of the dish. Do not allow the acidified water to come in contact with the lower surface of the oak leaves (surface with telia).

10. Repeat steps 8 and 9 for each dish. Place bottom of dish just filled with acidified water on top of stack before placing lid with leaves on top. This helps to eliminate sloshing acidified water on leaves.

11. Stack petri dishes from like inoculum sources up to 20 high. Keep Run A or Run B dishes together. Label the top dish of each stack with test number and run, inoculum source, and date of harvest.

12. Incubate dishes for 24 hours at room temperature (below 81 ^{o}F). Adjust air conditioning or furnace thermostats, if necessary, to maintain normal temperatures. Usual room temperature is set for 70 ^{o}F. Avoid direct sunlight, as basidiospores may be destroyed.

13. Return leftover leaves to bag with label and place in 5 °C incubator. Discard after spores have been filtered.

14. Repeat steps 2 through 10 for each bag of leaves.

Day 2: Collect Basidiospores (Filtering)

1. Take a glass millipore filter base with a fitted stainless steel millipore screen, wet with distilled water, place a silicon ring seal on screen, and place a glass millipore funnel on top of base. Clamp together with blue millipore clamp.

2. Pour acidified distilled water, which now contains basidiospores, from all petri dishes of one run through the screen into an Erlenmeyer flask. The screen traps insect larvae and other large particles that may have fallen from leaves. To remove spores that have settled on bottom of petri dishes, with a squirt bottle rinse the bottom of each petri dish lightly with distilled water. Add rinse water to the same flask.

   Label flask with test number, run, inoculum source, and date.

3. Use a millipore system to filter spores from the suspension in the Erlenmeyer flask. Set up filter funnels as follows:

   a. Place a ground glass millipore filter base into a 1,000-ml Kimac two-arm filter flask.

   b. Wet stopper of filter base and press down firmly to obtain a good seal.

   c. Wet top of base and place a filter pad of 3 µ pore size on top. Center pad with forceps.

   d. Place funnel on top of millipore filter base carefully without sliding it around.

   e. Place clamp between bottom of funnel and top of base.

4. Check attachment of filter flasks. Six flasks are attached in a series to one suction system. The suction system is composed of two lines--one attaching the tops of each flask and one attaching the bottoms of each flask. Running water provides the vacuum needed for filtration.

   Three flasks are used for Run A; the other three are used for Run B. Pads are kept separate by run.

5. Turn on water. If less than six flasks are used, place a clamp on the tubing between the last flask in use and the first flask not in use.

   If a flask is not being used between flasks that are in use, remove filter base and put in a stopper.

6. Pour 200 mL of basidiospore suspension into each funnel. The quantity depends on the density of spores in the liquid. If the suspension is light in color, the spore concentration may be low and 225 to 250 mL can be filtered onto one pad. If the suspension is darker, less volume should be added to prevent overcrowding of basidiospores on the pad.

Carefully observe filter setup as suspension is being poured. If the system leaks, stop pouring suspension, remove clamp, and try to reseat the funnel.

7. Clamp the upper line past the last flask in the series to begin suction. Draw off all liquid without letting pads dry. Rinse the sides of the funnel with distilled water as filtering takes place. Release vacuum by removing clamp from upper tubing line.

8. Add 25 to 50 mL distilled water from a squirt bottle to agitate the spores and rinse the sides of the funnel. Reapply suction and filter until excess liquid is removed. This procedure rinses any remaining acidified water from basidiospores.

9. Release the vacuum, remove millipore clamp, and carefully tilt the funnel sideways. Remove pad containing basidiospores with millipore forceps (flat tips).

   Apply a new pad to filter base following steps 3C through 3E. Repeat steps 6 through 9 for each pad until all suspension has been filtered.

10. Place two 125-mm filter paper disks in a 150-mm petri dish. Moisten disks with distilled water. Pour off any excess water. Place pads of basidiospores on moistened filter paper. Store disks containing a maximum of six pads at 5 °C. Basidiospores are normally used within 1 week of collection but can be stored up to 6 weeks.

11. Drain filtrate from Kimac flasks and turn off water. Check moisture of filter paper in dishes frequently. If moisture in the filter paper is low, add distilled water to the edge of the filter paper with a squirt bottle.

12. Wash funnel tops, bases, and petri dishes in dishwasher.

PREPARING PINE SEED FOR SCREENING

Prior to germination, pine seeds are placed in vials marked with a greenhouse code. Check seedlots are similarly placed in vials and marked. Loblolly seeds must be stratified 8 weeks before germination.

Preparing Seed for Planting and/or Stratification

1. Using a permanent ink marker, label two plastic vials (discarded Coulter counter accuvette) with test number and seedlot greenhouse code. Do this for each seedlot.

2. Place equal amounts of seeds for a seedlot (visual estimate) into each vial. Cap vials with snap-fit plastic lids. Check off seedlot just divided on the TEST TRAY TALLY sheet.

3. Place vials in a styrofoam accuvette holder.

4. Repeat steps 2 and 3 for each seedlot.

5. Remove jars of seeds from freezer of each check seedlot listed on the WORK SCHEDULE. Allow seeds to warm to room temperature before opening. (If this is not done, moisture content of seeds will increase when jar is opened. This decreases storage life of the seeds.)

6. Check Seed Inventory Book lists the weight of the number of seeds needed from each check lot. Repeat steps 1 through 3 for each check seedlot required for the test. Record appropriate information in inventory book.

7. Label each holder with the test number.

   Place holder in refrigerator at 5 °C until planting or stratification date, depending on species.

Stratifying Loblolly Pine Seed

1. Remove styrofoam holder containing vials of seeds from refrigerator. Remove lids from vials.

2. Fill each vial with enough distilled water to completely cover seeds.

   Replace cap and agitate. Place vial back in holder and return to refrigerator for 3 days.

3. After 3 days, remove holder from refrigerator. Remove cap from a vial. Cover vial with copper screen and drain off water.

   Replace cap and place vial back in holder.

   Repeat for each vial.

4. Return holder to refrigerator for 8 weeks.

GERMINATION OF PINE SEEDS

Seeds are surface-sterilized and placed in germination flats in the greenhouse. A mist system waters the germination flats, and supplemental lighting is provided if necessary.

Surface Sterilizing and Placement in Germination Beds

1. Remove seed vials from refrigerator. Allow to warm to room temperature (requires approximately 1 hour).

2. Remove the appropriate greenhouse test booklet from lab notebook. Transport the folder and seeds to the headhouse.

3. Fill one 4- by 6- by 2-inch germination tray per vial with No. 2 Attic grade vermiculite to within 1/2 inch of top of tray. Using a black, permanent ink pen, mark a white, plastic T-stake with the test number and greenhouse seedlot code shown on each vial. Place one tag in each germination tray. Place eight germination trays in one plastic, 19- by 15-1/2- by 2-inch flat.

4. Prepare a solution of 20 percent hydrogen peroxide (H_2O_2) by mixing concentrated H_2O_2 with tapwater in a 1,000-mL plastic, graduated cylinder. H_2O_2 and water proportions may vary with concentrations of H_2O_2 being used. (CAUTION: Rubber gloves should be worn when using hydrogen peroxide. Skin burns can occur.) Depending on test size, approximately 600 to 900 mL of 20 percent solution will be needed.

5. Remove lids from 10 seed vials. Pour enough 20-percent hydrogen peroxide solution into each vial to cover seeds. Soak seeds 10 minutes, stirring every 2 minutes.

6. Place a 1/16-inch mesh copper screen over a vial. Pour peroxide solution out, taking care not to spill any seeds.

   With screen still held firmly in place, fill and empty each vial with cold running tapwater three or four times. Shake out excess water and tap bottom of vial on counter to knock seeds towards bottom of vial. Repeat for the other nine vials.

7. Empty seeds onto vermiculite in germination trays. Allow seeds to air dry. Be extremely careful to match greenhouse code number on vial with greenhouse code number on plastic T-stake in germination tray.

8. After they have dried, spread seeds evenly over the vermiculite, being careful not to bury them. The tip of a plastic T-stake can be used for this purpose.

9. Repeat steps 5 through 8 until all seeds are in germination flats.

10. Water very carefully until they are thoroughly soaked. Do not wash vermiculite completely off seeds.

11. Place germination trays into mist bed. Group all trays in a test together.

    Check off seedlots placed in mist bed on the TEST TRAY TALLY sheet in the greenhouse test booklet and write your initials after the planting date.

    Mist bed automatically mists germination trays for 15 minutes twice daily with tapwater. Supplemental lighting is used to provide a minimum of 100-foot candles of light for 16 hours each day.

TRANSPLANTING PINES

Pine seedlings are transplanted as they germinate into 20-tree container holders labeled with a plastic tag. Transplanting begins approximately 10 days after planting. Preparation of planting medium is described elsewhere in this manual.

Preparing Tags

1. Label tags before germination begins. Use a different color tag for each run. Tags should indicate test number, run, greenhouse seedlot code, and tray number in the following format:

105 A - first three digits of test number plus run
81 - last two digits of test number
5 - greenhouse seedlot code
2 - tray number

In the standard operational design, there are three trays of each seedlot in each of two runs, plus one extra tray per seedlot. Check WORK SCHEDULE to see if the test deviates from this design.

2. Arrange tags in bundles by greenhouse seedlot code. Arrange tags within bundles by tray number to ensure that seedlings are represented equally in all runs.

Preparing Tubes for Transplanting After Filling in Soil Mixer

Preparation should be done the same day the tubes will be used. Preparation of the tubes for transplanting is a three-step process--packing the tubes, transferring tubes to metal trays, and fertilizing the tubes.

1. The large, black tube holders (98 tubes) are placed in one of the small carts and dropped several times until the potting medium is shaken as far down the tube as possible.

2. Fresh soil is put into the tubes by hand until the tubes are full.

3. The soil is pressed down for the first inch or so in each tube by using the hand-operated seven-tube packer (made at RSC).

4. The tubes are then transferred to the metal tray that holds 20 tubes each.

5. The trays are then put in another one of the small carts.

6. Tubes are then fertilized with a one-half concentration of Miracle-Gro--1 teaspoon per 2 gallons of water. It is best to use the water wagon for this. This would be 7-1/2 teaspoons per 15 gallons.

7. Tubes are continuously fertilized until the entire mixture is saturated.

8. Check for tube saturation by holding one tube up and squeezing it. Fertilizer should be dripping out the bottom.

Transplanting Seedlings

1. Transport prepared tubes and holders from soil building to headhouse.

2. Identify seedlots with at least 20 germinated seedlings.

3. Make 1/4-inch-diameter by 2-inch-deep planting hole in each tube of holder.

4. Choose a seedling at random from the appropriate seedlot. Carefully pull each seedling out of vermiculite and use thumb and index finger to gently wipe off any excess vermiculite. Starting at one end of the tray, place the seedling in one of the holes and press media lightly around seedling with fingertips to secure the seedling. Continue back and forth, from row to row, until entire tray is filled.

Plant each seedling deep enough to cover white root portion of stem. Discard any seedlings that are pale in color or pinkish, or that have a very crooked root. Continue only if an entire 20-tree holder can be planted.

5. Place appropriate tray tag in right corner of holder (facing you).

6. Cover medium with a thin layer of vermiculite.

7. Load all transplanted holders onto carts, move to glasshouse, and water thoroughly. (If the temperature in the greenhouse is above 80 ^{o}F, newly transplanted seedlings should be put in a shaded area of the greenhouse for 1 day.)

8. Repeat steps 1 through 7 each day until all tags have been used or until the scheduled transplanting cutoff date has passed. After all tags for a seedlot have been used, an extra tray of 20 seedlings should be transplanted. Make up a tag of a different color. Mark it with the test number, seedlot number, and "X." Put this tag in the extra tray. On the transplanting cutoff day, if fewer than 20 seedlings are available, transplant seedlings into a holder of at least eight seedlings. No seedling should be transplanted after the cutoff date.

Pine Transplant Final (PTF)

1. The final day for transplanting pines is exactly 3 weeks after the planting date. Check the test calendar and the test folder for this date.

2. Inspect all transplanted trees for dead or unhealthy ones.

3. Dead trees should be removed and replaced with healthy ones from the extra tray of the same seedlot.

4. Unhealthy trees should also be removed and replaced, if there are sufficient extras from the same seedlot.

5. Enough trees should be transplanted from each seedlot to refill each extra tray, or as many as possible.

6. Get test folder from notebook in headhouse.

7. Mark down the actual number of trees in each tray in Run A and each tray in Run B under the transplant (TR) column in the test folder.

8. This is a good time to doublecheck for dead or unhealthy trees. Check that all those needing to be removed and transplanted have been done.

9. After doublechecking, discard vermiculite and seeds from the germination trays.

10. Remove tray tags for trays that were not transplanted and put together by test on the shelf in the headhouse.

11. After inoculation, wash seedlot number tags and put them in a bleach bucket in headhouse.

12. Wash and sterilize germination trays, and put them back on the shelf.

PREPARATION OF PINE INOCULUM (MIXING DILUTION)

Basidiospore suspensions should contain 50,000 spores per mL for inoculating loblolly pine and 20,000 spores per mL for slash pine. Basidiospore numbers in suspensions are estimated to an accuracy of ± 3 percent with a Coulter counter. Basidiospores are washed into cold, distilled water, and the spore density is calculated by using an electronic particle counter. The desired basidiospore density and the total volume of dilution required are used to calculate the correct volume of water and stock dilution required. The resulting spore suspension is checked to see if it falls within the acceptable limits. The dilution for each run is mixed 1 day prior to inoculation.

Setting up Equipment

1. Turn on Coulter counter and allow to warm up for 2 hours before using.

2. Place repipetting jar of isoton (brown glass jar with pipette attachment) on magnetic stirrer and stir for a few minutes. (Stirring magnet is already inside jar.)

3. Place seven Coulter accuvettes (vials) per dilution in a holder.

4. Remove red, protective cap from tip of pipettor. Check calibration of volume to be dispensed (18 mL). Pipette approximately 50 mL of isoton from jar and discard. Check to see that no air bubbles are present in pipette system.

5. Fill each vial with 18 mL of isoton. Remove vial cap just before filling and replace immediately after filling. Reserve three of the seven vials for later use.

6. Rinse the following equipment in distilled water. Allow to drain. Equipment needed:

   a. 500 mL Nalgene plastic, graduated cylinder
   b. 100 mL Nalgene plastic, graduated cylinder
   c. 1,000 mL beaker
   d. 2,000 mL flask (preferably plastic) (1,000 mL flask, if dilution is less than 1,000 mL)
   e. 1 mL pipettor tip (green, plastic) for micropipettor
   f. funnel
   g. sieve screen (stainless steel, 3-inch diameter, 200-mesh)

7. Get a STOCK SUSPENSION SHEET (stock sheet - fig. 9) and an INOCULUM DILUTION SHEET (dilution sheet - fig. 10) from the bottom-right drawer of the microscope table.

8. Record date, inoculum source, test number, run, initials of person(s) preparing dilution, attenuation (normally two), aperture (normally four), and aeciospore germination percent (check form beside microscope).

   Note: Attenuation and aperture readings are taken from corresponding dials on Coulter counter.

9. Check WORK SCHEDULE on bulletin board for the test being mixed to obtain number of seedlots, inoculum density, and inoculum source.

FIGURE 9

INOCULUM DILUTION SHEET

Species & Source _____ Test No. _____
Run _____

Dilution Count

Vial 1	Vial 2	Vial 3
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

Sub Total _____ + _____ + _____ = Total _____

_____ - _____ = _____
average count — background count — adjusted count

_____ x _____ = _____ Within Limits _____
adjusted count — dilution factor — dilution density (spores/mL)

IF DENSITY IS NOT WITHIN LIMITS:

_____ x _____ = _____
calculated density — Vol. of dilution — No. spores in dilution

DENSITY TOO HIGH:

_____ - _____ = _____
No. spores in dilution — density required — Vol. required

_____ - _____ = _____
Vol. dilution required — Vol. present dilution — Vol. (mL) H_2O to add to dilution

DENSITY TOO LOW:

_____ - _____ = _____
total number spores required — No. spores in dilution — No. additional spores needed

_____ - _____ = _____
No. additional spores needed — stock density — Vol. (mL) stock suspension to add to dilution.

FIGURE 10

STOCK SUSPENSION SHEET

Test No. _____
Run _____

Date _____ Inoc. Source _____ Initials _____
Attenuation 2 Aperture _____ Threshold 10 Lamda 500
Stock Germination Before Inoculation _____% Dilution Germination After Inoculation _____% Aeciospore Germination _____%

Background Count Vial 1 _____
_____
_____ Aver. _____
\+ _____
Total _____

Stock Count

Vial 1	Vial 2	Vial 3
_____	_____	_____
_____	_____	_____
_____	_____	_____

Subtotal _____ + _____ + _____ = Total _____

_____ - _____ = _____
Aver. count — background count — adjusted count

_____ x 20 = _____
Adj. count — dilution factor — stock density (spores/mL)

(_____ x _____ mL) + _____ = _____
No. seedlots — No. trays — buffer — Vol. dilution required

_____ x _____ = _____
density req. — Vol. dilution req. — total No. spores required

_____ - _____ = _____
total number spores required — stock density — Vol. stock required

_____ - _____ = _____
Vol. dilution req. — Vol. stock req. — Vol. ice dist. H_2O req.

_____ + _____ = _____
Vol. stock req. — Vol. ice dist. H20 req. — Vol. dilution req.

10. Fill in number of seedlots, number of trays, buffer volume, and density required for the test.

11. Multiply number of seedlots by number of trays in run (3 for operational tests) by number mL per tray (10 mL per tray) and add volume of buffer (at least 200 mL). Result is the volume of dilution required for inoculation.

12. Multiply density required by volume dilution required. Result is the total number of spores required.

Mixing Stock Suspension

1. Filter 500 to 900 mL cold, distilled water from jug in 5 ^{o}C incubator through stainless steel screen into 1,000 mL beaker.

   Note: Volume dilution required determines volume of stock to prepare. Generally, a test requiring 1,000 mL or less to inoculate can be prepared from a stock suspension volume of 500 to 600 mL.

   1,000 to 1,400 mL of dilution = 700 to 800 mL stock suspension
   1,400 to 2,000 mL of dilution = 800 to 900 mL stock suspension
   Dilution volumes over 2,000 mL need special planning.

2. Rinse one to four pads (depending on stock volume being mixed and density of spore pads) of basidiospores through stainless steel screen into beaker of distilled water from step 1. Do this by laying one pad at a time (held with forceps) in stainless steel screen and squirting distilled water over pad of spores.

3. After all spores have been rinsed into beaker, rinse screen with 70 percent alcohol, tapwater, and distilled water, and set aside for later use. This dilution will be referred to as "stock."

4. Place stirring magnet in stock and place on magnetic stirrer. Stir vigorously for a few seconds, then set stirring speed between 4 and 5.

5. Place (1 mL) green pipetting tip on micropipettor. Draw a sample of stock and dispense back into stock. Repeat two or three times.

6. Draw 2 mL of stock (1 mL at a time) and dispense into each of three vials of isoton (from step 5 of Setting up Equipment).

7. Cover stock beaker with foil. Mark date, test number, run, inoculum source, and the word "STOCK" on foil with felt marker. Place stock in 5 ^{o}C incubator.

8. Using Coulter counter, take counts on the above three vials plus one vial of plain isoton. Instructions for using the Coulter counter are included in a separate section entitled "Coulter Counter Operation."

9. Record counts of vial with isoton only for the background count. Record four counts, then calculate the average background count.

10. Record the average background count on STOCK SUSPENSION SHEET and on the INOCULUM DILUTION SHEET.

11. Record four counts for the first vial, the second vial, and the third vial. Calculate subtotal for each vial. Calculate total for all three vials, and divide by 12 for the average count. Subtract background count from average count. Multiply this adjusted count by 20 (dilution factor). The result is the stock density (spores/mL). Stock counts of 10,000 spores/mL or higher should be diluted with distilled water and recounted before mixing dilution.

12. Divide the total number of spores required by the stock density to obtain the volume of stock required.

13. Subtract volume stock required from volume dilution required. The result is the volume of cold, distilled water required.

14. Recheck all calculations.

Mixing Dilution

1. Remove stock suspension from incubator and stir as in step 4 above.

2. Use Nalgene graduated cylinders to measure volume of stock required and pour into a flask. Volumes of 199 mL or less should be measured in the 100 mL cylinder. Volumes of 200 mL or more are measured in the 500 mL and 100 mL cylinders in increments of 100 mL. For example, if 340 mL of stock are required, measure 300 mL in the 500 mL cylinder and pour into a flask. Measure the remaining 40 mL in the 100 mL cylinder and pour into same flask.

3. Measure volume of cold, distilled water in the same manner and add to flask containing the required volume stock. Filter cold, distilled water through stainless steel screen as it is added to the flask.

4. Remove stirring magnet from stock suspension and place in dilution.

5. Place dilution flask on magnetic stirrer. Stir vigorously for a few seconds, then set stirring speed between 4 and 5.

6. Spray one petri plate of 2 percent water agar with leftover stock. Do this by placing the tube of a Power-Pak hand-held sprayer into stock and hold petri plate open in front of spray. Mark petri plate with test number, run, date, and the word "STOCK" with a felt marker. Incubate plate at room temperature for 24 hours.

7. Return leftover stock to 5 ^{o}C incubator.

8. Use remaining three vials of isoton. Using micropipettor, add 2 mL of dilution to each vial. (Same steps as for stock vials.)

9. Place stopper in dilution flask. Label flask (with masking tape) by marking test number, run, inoculum source, date, and the word "DILUTION."

10. Place dilution flask in 5 ^{o}C incubator.

11. Count each vial using Coulter counter. Record counts on INOCULUM DILUTION SHEET. Use same counting technique as for stock.

12. Calculate dilution density. If density is within ± 3 percent of required density, mark "within limits?" as yes. For example, density of 20,000 must fall between 19,400 to 20,600.

Dilution Density Not Within Limits

1. Perform calculations on lower portion of INOCULUM DILUTION SHEET, if density is not within limits.

2. If density is not within limits, multiply the calculated density by the volume of the present dilution. The result is number of spores in dilution.

3a. DENSITY TOO HIGH: Divide the number of spores in dilution by density required. Result is new volume of dilution required. Subtract present dilution volume from new dilution volume. Result is volume of cold, distilled water to be added to present dilution.

Caution: Calculated volume of water to add to dilution is generally too high. Reduce amount by about 5 mL.

3b. DENSITY TOO LOW: Subtract actual number of spores in the dilution from total number of spores originally required (recorded on stock sheet). The result is the number of additional spores required. Divide this number by stock density. This result is the volume of original stock to be added to present dilution. Add additional stock to dilution.

4. Draw three new vials of isoton and repeat procedure for counting dilution. Record counts on new INOCULUM DILUTION SHEET.

Final Steps

1. Staple all sheets together and place beside microscope.

2. After germination percentages have been appropriately recorded, place sheet in appropriate test folder in office master files.

3. Repeat "Mixing Dilution" process on day 2 for run B.

Coulter Counter Operation

1. Turn power switch to on. Allow counter to warm up for 2 hours, if possible.

2. Turn top vacuum control stopcock one-quarter turn in either direction.

3. Numeric readout should reset to 8888, then 0. If this does not occur within a few seconds, turn stopcock another one-quarter turn. Wait a few seconds and try again.

4. Agitate vial to be counted by shaking 10 times. Carefully remove cap.

5. Lower white vial platform of counter and remove vial from platform. Note: Vial platform is spring-controlled; do not let go while lowered. Touch vial to tip of glass tube (manometer) to remove excess drops of isoton. Do not attempt to remove vials unless counts are completed or vacuum control stopcock is closed.

6. With platform still lowered, place vial to be counted on platform. Background vial should be counted first. Line vial up with black square on platform. Gently raise platform.

7. Obtain numeric readout by turning the top vacuum control stopcock one-quarter turn in either direction. Reset after each count. A count begins soon after stopcock is closed. Counts are registered in numeric readout. Each count takes approximately 14 seconds. There are two screens at top-left portion of counter. The left screen is an electronic oscilloscope which indicates pulse pattern of cells or particles passing through the aperture. The right screen is a projection of the aperture. Periodically make checks for debris in the aperture. If blockage occurs, allow count to finish, lower platform, brush aperture with counter brush, raise platform, and disregard the next two counts. Record the next four counts on appropriate form. The Coulter counter manual contains additional information on use of this machine.

8. Repeat steps 4 through 7 for each vial to be counted. Place vial of pure isoton back on counter after all counts are completed.

PINE INOCULATION (PI)

Inoculation of an operational (normal) test requires various procedures spanning a 5-day week. Check the calendar to make sure which test is to be inoculated that week, and also check the dates on the test folder. Read all of the following steps before beginning, then follow the steps for each day.

Day 1:

1. Get the test folder and the list of "number of trees per tray" from the headhouse. Also, get tray tags not used during transplanting.

2. Inspect both the "A" and "B" runs for dead or unhealthy trees.

3. If each of the six trays for each seedlot has 20 trees, then no adjusting is needed.

4. If extra trees are available (from the extra tray) from the same seedlot, replace the dead and unhealthy trees with these. If not enough extra trees are available, do not remove the unhealthy trees, but do remove the dead ones.

5. If any of the six trays in any seedlot are not full, remove all six trays and set them apart from the other trays.

6. For each seedlot that does not have six full trays (120 trees), follow these steps:

    a. Find the total number of trees in that seedlot.

    b. Find the total number of trees on the list of "number of trees per tray" and distribute the trees according to the list. Use the tags left over from transplanting, if needed.

c. Record the adjusted (correct) number of trees for each tray in test folder.

7. After inspecting and adjusting each tray, record in the test folder, under the inoculation column (IN) on the TEST TRAY TALLY sheet, the number of trees for each tray in each seedlot. This includes trays 1, 2, and 3 in the "A" run and trays 1, 2, and 3 in the "B" run.

8. When all trays have been adjusted and their numbers recorded, water all of the "A" run trees whether they need it or not.

9. Load all of the "A" run trays on carts and group them together (tray 1's together, tray 2's together, and tray 3's together).

10. To make sure all trays are loaded, mark each off with a check under column (IN) on the TEST TRAY TALLY sheet in the test folder as it is loaded.

11. Leave the "B" run trees on the benches where they are.

12. Move all the "A" run trees (now loaded on carts) into the headhouse, where they remain overnight.

13. The headhouse doors must remain closed, unless the outside temperature is at least 65 ^{o}F. The door must be closed if it is raining or very windy outside.

Day 2:

1. Plug in inoculation rig and fill reservoir beaker, located on magnetic stirrer, with screened distilled water (use a 3-inch by 100-mesh screen). Turn rig on so that rig is pumping only water. Run for at least 30 minutes before checking pump rate.

2. Check water bath behind chamber to see if it is full.

3. Check water bath temperature; it should be at 20.5 $\pm$ 0.5 ^{o}C. If temperature is not correct, move heat thermostat up or down one mark at a time and check at 30-minute intervals.

4. Read stock germination petri dish in the rust lab with microscope and record percent germination on the STOCK SUSPENSION SHEET. Get test dilution from incubator in rust lab, and place on magnetic stirrer. Make sure magnet is spinning, not jumping.

5. Get two 2 percent water agar petri dishes from incubator. Label one with the test number, date, and "lab." Label the other with test number, date, and "chamber."

6. Wrap both dilution flask and petri dishes with aluminum foil and take them back to the headhouse.

7. Put both flask and petri dishes in refrigerator until inoculation.

8. Each nozzle on the inoculation rig should pump 30 mL of water per 60 seconds.

9. Check this pump rate by placing the 50 mL graduated cylinder under one nozzle at a time so that all water from that nozzle is pumped into it.

10. With the water being pumped into the cylinder, start the stopwatch when the level reaches 10 mL.

11. Observe the stopwatch, and when exactly 30 seconds have ticked off, switch off the inoculation rig.

12. Check volume in the graduated cylinder. It should be at 25 $\pm$ 0.5 mL.

13. Check each nozzle three times.

14. If volume of water is incorrect, adjust corresponding nozzle pump. This is done while pumping water by increasing or decreasing the dial one increment at a time. Recheck volume being delivered after each adjustment. Disregard scale on pump, as it is inaccurate. If problem continues, nozzles should be removed, disassembled, and cleaned by spraying them with compressed air and/or cleaning the small opening with a thin wire.

15. Check inside of chamber to see that water is dripping down the sides.

16. Check to see if mist comes out of gutter pipe overhead. This is the humidifier.

17. Use the stopwatch to check if humidifier runs for 20 seconds every 2 minutes.

18. Turn on conveyor belt and check speed. The belt should be moving at a rate of 1 foot every 10 seconds. Turn belt off.

19. Plug in air compressor and turn it on. Open valve at bottom of air tank and drain the water.

20. Hook air line to inoculation rig and turn rig on. Air pressure gauge should stay constant at 12 lb/in^2. Wait at least 5 minutes before adjusting.

21. Air pressure can be adjusted with knob at gauge.

22. Mist pattern from nozzles should be adjusted in such a way that all trees are covered as equally as possible. Use extra trays of trees for this purpose.

23. To move nozzle, loosen with Allen wrench and move up or down, forward or backward. Both nozzles should be moved equally.

24. Get test folder from headhouse.

25. INOCULATION SHEET from folder must be filled out, including:

    a. Run "A" or "B"

    b. Date

    c. Time inoculation begins and ends

d. Temperature at inoculation rig at beginning and ending of inoculation

e. Calibration: (Nozzle 1) Position 1: Pump rate at beginning and end
(Nozzle 2) Position 2: Pump rate at beginning and end

f. Weather conditions: general description of outside conditions

g. Inoculation tray order: tray 1's, tray 2's, tray 3's, or 3's, 2's, 1's, etc. (not seedlot numbers).

26. Make sure carts of trees are in such a position that they will be easy to move and that they are in a reasonable order (tray 1's together, tray 2's together, and tray 3's together).

27. Lock headhouse doors and hang inoculation sign in door window.

28. Close door to bay 2 and turn fans off in bay 1.

29. Plug humidifier into untimed outlet (number 16).

30. Close white curtain to block sunlight from inoculation area.

31. Get dilution flask and petri dishes from cooler.

32. Vigorously shake dilution flask, being careful not to drop it. There is a magnetic stirring bar inside of the flask.

33. Make sure no stands, flasks, beakers, trays, etc., obstruct the spray pattern from nozzles.

34. Put someone inside chamber.

35. Pull down curtain over chamber door while door remains open during inoculation.

36. Move inoculation rig into opening in curtain.

37. Empty water out of inoculation rig reservoir.

38. Again, shake dilution flask and pour into rig reservoir.

39. Turn rig on and make sure magnetic stirrer is working.

40. Place dummy tray first on conveyor. This is a tray of extra trees.

41. When dilution has started to spray from nozzles, start the conveyor.

42. Begin placing test trays on conveyor, making sure the tray tag is in the back-left corner, away from the chamber, and that the tag is turned perpendicular to the direction of travel in order to block a minimum of mist from the nozzles. Leave no space between trays. Place a dummy tray last in line.

43. Take each tray of trees off the conveyor only after it has reached the end of the conveyor belt.

44. Gently place each tray on a shelf inside the chamber. Be sure all tray 1's are together, tray 2's are together, and tray 3's are together.

45. When the last tray has been taken off the conveyor, turn the conveyor off.

46. Take the petri dish labeled "chamber" and spray it with the mist coming from either or both nozzles for approximately 30 seconds. Set it inside the chamber with the lid ajar. Do the same for the petri dish labeled "lab," but rewrap it with foil and put it on the counter in the rust lab.

47. Pull inoculation rig out of chamber door and let person out. Close door and make sure light is off.

48. Record time and temperature on INOCULATION SHEET.

49. Check air pressure and record it on INOCULATION SHEET, then disconnect air line.

50. Pour remaining dilution from reservoir back into flask, rewrap in foil, and put back in incubator in lab.

51. Rinse out reservoir with screened distilled water, then fill with same.

52. When water reaches nozzles, check pump rate and record on inoculation sheet.

53. Plug humidifier back in timed outlet. Open and unlock doors, open curtain, turn fans back on, and remove inoculation sign.

54. "A" run trees remain inside of chamber for 18 to 24 hours and will be removed on day 3 (Wednesday).

55. Water all "B" run trees and load on carts, keeping all tray 1's together, tray 2's together, and tray 3's together.

56. Repeat steps 8 through 12 of day 1.

Day 3:

1. Before removing trees from chamber, plug humidifier into full-time outlet (number 16). If sun is shining through glass, close white curtain while unloading chamber.

2. Remove "A" run seedlings from chamber only after they have been inside the chamber for 18 to 24 hours.

3. Very carefully unload trays onto cart so as not to shake off water droplets.

4. Remove petri dish to read later.

5. Record time and temperature of tree removal from chamber on inoculation sheet.

6. Move inoculated trees, now loaded on carts, into headhouse and observe all door rules.

7. Doors and screens on doors will always be closed until water droplets have air dried on needles.

8. Doors and screens on doors will always be closed if direct sunlight can hit trees.

9. Before inoculating "B" run, read run "A" percent germination of basidiospores on chamber petri dish and record on "A" run STOCK SUSPENSION SHEET. If germination is low (less than 75), read run "A" lab petri dish.

10. Read stock suspension petri dish in lab and record on "B" run STOCK SUSPENSION SHEET.

Day 4:

1. Move "A" run trees to one of the plastic houses (see test folder to find which one).

2. Record time and outside temperature of move on INOCULATION SHEET in test folder.

3. Try to put all trays on the same bench and in order (1's, 2's, and 3's).

4. Move trees only if:

   a. Outside temperature is at least 40 ^{o}F.

   b. It is not raining.

   c. It is not windy.

5. If trees cannot be moved, they should be put back in glass house only until they can be moved to plastic house.

6. Unload "B" run from chamber as done for "A" run, except for the following changes:

   a. Humidifier does not need to be on full time.

   b. There will not be a stock suspension petri dish to read.

7. Make large tags to put on both ends of test after it is in the plastic house. They should have test number, date of first fertilization (PF), and "do not fertilize until the (PPF) date."

Day 5:

1. Move Run B trees to plastic house with Run A trees.

2. Follow same instructions for moving Run B as done for Run A on day 4.

3. Make sure test tags are at both ends of test.

4. Put test folder in notebook in plastic house.

PREPARING ARTIFICIAL PINE PLANTING MIX

The pine medium is mixed in a 1-cubic-yard Bouldin and Lawson twister mixer, model number 121. The mixer is swept clean before mixing. The medium is a 3-4-3 mix of peat moss, vermiculite, and perlite.

Ingredients

1. One 6-cubic-foot bail of sphagnum peat moss (Fulson Sunshine)
2. Two 4-cubic-foot bags of No. 2 vermiculite (GSA)
3. One 6-cubic-foot bag of coarse perlite (Terra-Lite)
4. Water

Mixing Procedure

1. Sweep old media from mixer and turn on exhaust fan.
2. Using electric hoist, place peat moss on top of mixer and empty into mixer.
3. Mix peat moss for 15 minutes only.
4. Add both bags of vermiculite.
5. Mix for exactly 3 more minutes.
6. Add perlite to mix.
7. Mix for exactly 3 more minutes.
8. Add water to the mix by opening the valve on the white pipe on top of the mixer.
9. Run the water for about 3 minutes while the mixer is running.
10. Turn off the mixer and check the amount of moisture in the medium by compressing a handful of medium and tossing it up 6 to 8 inches and let it hit in your hand. The correct consistency would be for the compressed medium to take two to three tosses to break apart.
11. Add more water, if needed, and recheck moisture level. Be careful not to get the medium too wet.
12. Never run the mixer more than necessary.
13. After water has been added to the mix, it should be used within 2 weeks or discarded.

Operation of Soil Mixer to Fill Tubes

1. About 30 black holders of clean Ray Leach tubes (98 tubes) should be ready beside the mixer.
2. Two people are required to run the filling operation.

3. One person feeds the tubes onto the conveyor, and the other lifts them off and adjusts the flow of medium.

4. The trapdoor should be about three-fourths open.

5. The conveyor speed should be set as slow as possible.

6. The roller/packer should be set to pack the medium to about 1 inch from the top of the tubes.

7. Turn on the return, table, and conveyor.

8. Turn on the mixer and open the trapdoor.

9. Tube holders should be put in one behind the other, and a small amount of pressure should be applied.

10. When removed, the holders should be stacked with plywood shelves between side-by-side rows of three holders.

11. Stacks of holders should be covered with plastic, when not being used, to prevent moisture loss.

FERTILIZATION AFTER INOCULATION (PF) AND (PPF)

The first fertilization after inoculation (PF) is done 3 weeks after the inoculation date (any day of that week). The day seedlings are fertilized should be a day when they need to be watered. The fertilizer, 59.2 tablespoons of Miracle-Gro per 100 gallons of water (see Injector Operation), should be applied until the tubes are totally saturated.

The second fertilization of a test can occur on or after the programmed pine fertilization (PPF) date. When a test reaches this date, it is included in the regular (every 6 weeks) fertilizing schedule. The test can be fertilized any day of the same week that regular fertilizing is scheduled. The test should be fertilized on a day when seedlings need to be watered. The fertilizer, 59.2 tablespoons of Miracle-Gro per 100 gallons of water should be applied until the tubes are totally saturated. When fertilizing, it is easiest to use the "GEWA" fertilizer injector (see Injector Operation).

Both the (PF) and (PPF) dates are in the greenhouse test folder. The folder should be checked before doing any fertilizing.

If it is time for the 6 weeks' fertilization and less than 21 days has passed since the first fertilization date (PF) of a test, DO NOT fertilize. If 21 (PPF) days or more have passed, then that test is included in the 6 weeks' fertilization.

"GEWA" Fertilizer Injector Operation

1. The injector will hold 3 gallons of concentrated, dissolved Miracle-Gro fertilizer. This concentrate is enough for 300 gallons of fertilizer. The injector ratio is preset at 1:100. The concentrate is held inside the injector in a rubber bladder. As tapwater flows through the injector, the bladder is squeezed and a very small stream of concentrated fertilizer is added to the tapwater as it exits the injector.

2. Do not mix concentrate in the injector.

3. Mix concentrate 3 to 4 hours prior to use, if possible.

4. In a plastic container marked "for injector" (located in fertilizer storage refrigerator), fill to mark or measure 59.2 tablespoons of Miracle-Gro and dissolve in 1 gallon of hot water. Mix 2 gallons at a time in bucket.

5. Once mixed, allow enough time for sand in fertilizer to settle out.

6. After sand has settled, cover opening of injector with four layers of cheesecloth. Pour concentrate into injector through cheesecloth.

7. Close top of the injector.

8. Close valve on the bottom of the injector.

9. Hook up hose to tapwater and outlet hose to top of the injector. Note direction arrow on connection.

10. Turn water on.

11. Open top of injector slightly to allow air to escape, then reclose.

12. Check periodically to see that fertilizer is still being dispensed by spraying water into a white cup. The water should be blue.

13. When injector is empty (watch for color to fade), disconnect water lines. Empty water from around bladder by opening valve at bottom.

14. If fertilizer is not being dispensed and bladder is not empty, then remove dispensing valve and strainer and clean (see Operating Manual in Files).

ANALYZING SCREENING TEST DATA

Data files are created using the Record Keeper program. Data are entered into the computer in the greenhouse. Data analysis is then performed and a report prepared.

Data File Creation

1. Remove Record Keeper diskette from file box and place in disk drive 1.

2. Place an initialized or previously used storage diskette in disk drive 2.

   Note: Consult computer manual for instructions on initializing an unused disk. Several data-base files can be stored on a single disk.

   CAUTION: Initializing a disk will ERASE any previously stored information.

3. Turn on computer. Program will automatically load.

   If computer is already on, turn off and then back on or simultaneously press the Control, open-apple, and reset keys.

Note: If typing errors occur, use left arrow (<--) to backspace to error, type correction, and continue or follow program directions to exit from various steps.

4. Select program "1. RUN" from the Utilities Program menu.

5. Select program "1. Define Data Base" from the Record Keeper menu and press return.

6. Enter name of data base.

   Note: If using a disk on which data have already been stored, a catalog of files already on the storage disk will be displayed. Duplicate names are not allowed.

   Operational test data-base names begin with the letters OP, followed by the test number; example, OP10190. Research names begin with R and the test number. Developmental names begin with D and the test number.

7. Enter the field names. Press return after each name is correctly entered.

   Corrections can be made before pressing return by using the left arrow key (<--) to backspace under the error. Retype name or letter(s) and continue.

	Slash	Loblolly
Field #1	PREFIX	PREFIX
2	GH CODE	GH CODE
3	Tray Number	Tray Number
4	Tot Trees	Tot Trees
5	SYMNOS	LEFTOVERS
6	GALLS	GALLS
7	ROUGH	SMOOTH
8	LT25mm	FAT
9	HEALTHY	(not used)

8. Press return to quit after all fields are entered.

9. Correct Y/N? If correct, answer Y (without return); if not correct, press N (without return) and repeat step 7.

10. Enter field lengths and types. Press return after each correct response.

Field	Length	Type	Decimal Places
PREFIX	16	A (ALPHA)	
GH CODE	2	A	
Tray Number	1	A	
Tot Trees	2	N (NUMERIC)	0

	Slash	Loblolly		
SYMNOS OR LEFTOVERS	2	4	N	0
GALLS	2	4	N	0
ROUGH OR SMOOTH	2	4	N	0
LT25mm OR FAT	2	4	N	0
HEALTHY	2	4	N	0

11. Correct Y/N? is asked after each field entry. Answer Y (without return) if correct. Answer N (without return) if not correct; computer will ask again for field length, type, etc.

12. DO YOU HAVE COMPUTED FIELDS (Y/N)? Answer Y.

13. Fields will be displayed with a variable code. Enter formula as follows:

    Slash

    F09 = F04 - F05 - F06 which is

    HEALTHY = TOT TREES = SYMNOS - GALLS

    Loblolly

    F05 = F04 - F06 which is

    LEFTOVERS = TOT TREES - GALLS

    Answer CORRECT Y/N?

14. Computer will record information on a directory file and will create a data file on the storage disk in disk drive 2. Program then returns to the Record Keeper menu.

15. If data entry is to occur at a later time, select program "12. Quit" and press return. Select program "9. Quit" from Utility Program menu.

    Note: If data entry immediately follows defining data base, then select program "2. Data entry" from Record Keeper menu and press return. Follow steps in DATA ENTRY procedure.

Data Entry

1. Set up Apple IIc computer and monitor in greenhouse next to test being read. An extension cord and multiple plug outlet are required to supply electricity.

2. Insert Record Keeper disk in disk drive 1.

3. Turn on computer and monitor. Program will automatically load. If disk is inserted after computer is turned on, then press Control, open-apple, and reset simultaneously.

Note: If not using greenhouse copy of Record Keeper, check Record Keeper Configuration program; IIc configuration calls for one disk drive and no printer.

4. Select #1-RUN from Utility Program menu. Do not press return.

5. Select #2-Enter Data from Record Keeper menu and press return.

   Note: Computer responds with "Insert data-base diskette in drive 1 and press any key."

6. Remove Record Keeper disk and insert data-base (storage) disk. Press any key.

   Computer will display a catalog of files on the data-base disk.

7. Enter the data-base name (example - OP10990) and press return.

8. Answer the following computer questions:

Computer	User
Do you want automatic returns when fields are completely filled (Y/N)?	Y (no return)
Do you have any fields which have constant values (Y/N)?	Y (no return)

   (Field numbers and names are displayed.)

9. Enter number of the field which has a constant value. Enter 1 (Prefix) and press return. Enter 3 (tray number) and press return twice. The second return ends the sequence.

10. Computer then asks if "Fields having constant values correct (Y/N)?"

    If values are correct, press Y without a return. If values are not correct, press N without a return. If N was pressed, step 9 will be repeated.

11. Program proceeds to actual data entry.

12. Program displays field 1 and allows data to be entered. After initial entry of data for fields 1 and 3, automatic entries will be made for these two fields by the computer. Changes to these fields can be made with the edit function, which is explained later. Entries for all fields are as follows:

Assessing Seedling Response to Infection

Computer	User
1. PREFIX ^^^^^^^^^^^^^^^^	Prefix includes: Cols. 1-2 Operational year 3-7 Test number 8 Species code 9-10 Number of runs in test 11-12 Number of trays/seedlot/run 13 Reading number 14-15 Months at reading 16 Run (changes with each run) Ex. 1710190102031061
2. GH CODE ^^	Actual greenhouse code on tray tag
3. TRAY NUMBER ^	Actual tray number (changes with each set of trays)
4. TOT TREES ^^	Actual number of trees in tray. Dead trees should be excluded. Enter 0 in fields 4 through 8 for all missing trays.

Read individual trees. Tally total Symnos, galls, etc., on pushbutton tally counter for tray. Refer to report No. 82-1-5, November 1981, "HOW TO IDENTIFY SIX DIFFERENT FUSIFORM RUST SYMPTOM TYPES ON SEEDLINGS AT THE RESISTANCE SCREENING CENTER."

5. SYMNOS ^^	Total trees with symptom no swelling if reading slash.
LEFTOVERS	Entered by computer, if reading loblolly.
6. GALLS ^^	Total trees with galls.
7. ROUGH ^^ SMOOTH ^^	Depending on species, enter appropriate total.
8. LT25mm ^^ FAT ^^	Depending on species, enter appropriate total.
9. HEALTHY	Entered by computer, if reading slash.

13. Check entries. If corrections are needed, press E for edit. Computer will ask for field number of incorrect data: Enter FIELD NUMBER, press return, make correction, and press return. Do this for all fields needing correction. Press return without entering a field number to terminate sequence. If all data are correct, press S=Save.

14. Repeat step 12 until all trays are read.

Note: Fields 1 and 3 will automatically be entered for each record. Whenever run number changes and/or tray number changes, the E=Edit command should be used to make appropriate data changes before saving. Once changes are made to these fields, the new entry will be used until changed again.

15. After last tray is read and data are entered and saved, press ESC to end data entry.

    Computer will request that Program diskette be inserted in drive 1 and press any key.

16. After inserting the program disk, the Record Keeper menu will be displayed. Enter "12. Quit" and press return.

17. The Utilities Program menu will be displayed. Enter 9. Program is terminated.

    Note: If reading of test is not completed in one sitting, repeat entire procedure at each subsequent sitting. Program will automatically begin new data entry (record #) where last record ended.

18. After reading of test is complete, DATA ANALYSIS is performed.

Data Analysis

1. Remove disk labeled RSC ANALYSIS SLASH or RSC ANALYSIS LOBLOLLY and insert in drive 1.

2. Place disk containing data to be analyzed in drive 2.

3. Turn on computer. Program will automatically load.

   If computer is already on, turn off and then back on or simultaneously press the Control, open-apple, and reset keys.

4. Computer will respond "ENTER OUTPUT FILE NAME OR PR FOR PRINTER":

5. Enter PR. Press return.

6. Computer will respond with instructions which are self-explanatory and ask "ENTER THE NAME OF THE DATA FILE."

7. Enter the data set name. Ex. OP10190. Press return.

8. Computer gives information pertaining to the data set, then proceeds with questions for page-heading information.

   Answer questions accordingly. Use (<--) left arrow to backspace if typing corrections are needed. Press return after each response is entered.

   Computer will display your input and ask "CORRECT Y/N" after each response. If correct, press Y. If not correct, press N, make correction, and proceed.

   Answers for page-heading information are found on the TEST TRAY TALLY and/or WORK SCHEDULE form in the greenhouse folder.

a. ENTER CLIENT NAME (MAX 67 CHARS). Enter and press return.

b. ENTER INOCULUM SOURCE (MAX 28 CHARS). Enter and press return.

c. ENTER INOCULUM DENSITY (MAX 11 CHARS). Enter 20.0M SP/mL for slash or 50.0M SP/mL for loblolly (M = thousands and mL = milliliters).

d. ENTER INOCULATION DATES FOR EACH RUN IN MONTH/DATE/YEAR FORMAT (I.E., MM/DD/YY).

   ENTER DATE FOR RUN A: . Enter date and press return.

   ENTER DATE FOR RUN B: . Enter date and press return.

e. ENTER DATES OF READING FOR EACH RUN IN MONTH/DATE/YEAR FORMAT (I.E., MM/DD/YY).

   ENTER DATE FOR RUN A: . Enter date and press return.

   ENTER DATE FOR RUN B: . Enter date and press return.

9. After all page heading information is entered, computer asks "DO YOU WISH TO SEE THE INPUT INFORMATION LISTED (FOR PURPOSES OF EDITING)?

   ENTER Y/N (YES/NO)

   If response is yes, computer repeats step 8. If response is no, computer continues with entry of seedlot names.

10. Follow computer instructions for seedlot name entry. Seedlot names are entered in numerical sequence.

    Note: The first time a data set is analyzed, the seedlot names must be entered from the keyboard (K). Seedlot names are listed on the TEST TRAY TALLY form. Seedlot #1 corresponds to the seedlot name for GH CODE 01, etc. The computer will automatically create a file containing these names. If the data set is analyzed again, seedlot entry can be from disks (D).

11. Computer automatically ends seedlot entry procedure after last seedlot is entered. Seedlot names are displayed on the screen and the computer asks:

    DO YOU WISH TO EDIT ANY OF THE ENTRIES?

    ENTER Y/N (YES/NO)

    If yes, computer will ask for GH CODE, followed by a /, followed by the correct seedlot name. If no, computer proceeds to entry of check seedlots.

12. Enter number of check seedlots in test and press return. Review WORK SCHEDULE and TEST TRAY TALLY forms for number and GH CODES of check seedlots.

    Computer displays number entered and asks if correct.

13. Computer asks:

    ENTER EACH GH CODE SEPARATELY, FOLLOWED BY A (,) THEN R FOR RESISTANT, I FOR INTERMEDIATE, S FOR SUSCEPTIBLE, OR U FOR UNKNOWN. Press return after each check seedlot is entered.

14. Computer displays check seedlot names and type for verification. Make corrections if needed by following computer directions.

15. Computer asks:

    DO YOU WANT THE DELETED RECORDS LISTED?

    ENTER Y/N (YES/NO)

    If yes, seedlots not included in at least one run or missing will be displayed. If no, program proceeds.

16. Computer screen displays DATA INPUT CURRENTLY IN PROGRESS and disk 2 will be in use until all data are read.

17. Program will then calculate an INDEX OF RELATIVE RESISTANCE, followed by various tables for the GALLED symptom. This occurs for both species.

18. When printing of tables is completed, computer asks if you want to delete any seedlots and run the analysis again. Answer yes only if there was a SEEDLOT X RUN interaction shown in the analysis of variance table. The computer will suggest which seedlot(s) to delete and rerun the analysis.

19. Respond NO. Computer will then ask if other variables (symptoms) are to be analyzed. Answer yes.

20. If slash is being analyzed, select the A (all) option to get an analysis of all remaining symptoms. If loblolly is being analyzed, select 2-press return then 3-press return for SMOOTH and FAT symptoms then Q for quit.

21. Computer will perform all analysis requested and print the results.

22. After the last page is printed, program terminates automatically. Refer to step 1 if additional work is to be done.

23. Remove disks from drives, insert in covers, and place in storage box.

REPORT PREPARATION

1. A field office report is prepared from various tables for the analysis.

2. Forms from the greenhouse folder are incorporated in the office file.

3. Copies of the final report are stored in the office file and the RSC library.

4. Seedlings are discarded after data are checked and the analysis completed, unless the client had requested shipment of leftover seedlings. (See section on discarding seedlings elsewhere in this manual.)

5. Test records are stored indefinitely for future reference.

6. Computer printouts are stored in data files in the office.

DISPOSING OF SEEDLINGS AFTER SCREENING

After screening a test, healthy seedlings--those with no symptoms or with symptoms but no swelling--are shipped to clients at their request. Galled or unrequested seedlings are killed to ensure that the fungus is not spread outside the Resistance Screening Center.

Preparing Seedlings for Shipment

1. Place holder of each family from all runs together.

2. Select nongalled seedlings from all trays of each family, one family at a time. Both seedlings with no symptoms and seedlings with symptoms but no swelling should be selected.

3. Place the selected seedlings from one family into a plastic bag, roots first, with some moist peat moss around roots.

4. Place an I.D. tag in each bag to identify the family.

5. Pack bagged seedlings carefully into ice chest.

6. Place address labels on each chest to be shipped, and secure lids of each with strong tape.

   Note: If seedlings are stored outdoors between May 15 and October 1, all soils must be treated with granulated chlordane before shipping (Plant Protection and Quarantine regulations).

Discarding Pines

1. Mark seedlings to be discarded with plastic flagging ribbon. Do not water flagged seedlings.

2. Remove tags from all holders.

3. Pull seedlings from tubes and place in trailer attached to tractor.

4. Squeeze empty tubes and discard those that break.

5. Place good tubes in black 98-tube holder.

6. Haul seedlings to appropriate place and dump.

7. Stack empty holders and tubes in an appropriate location.

Sterilization of Containers

All plant containers are washed, sterilized, and reused whenever possible. This includes oak pots, seedling tubes, and germination trays, both large and small.

1. Oak pots and germination trays are first washed clean with soap and water. Washing is easiest if the medium is not permitted to dry before washing.

2. Pots and germination trays are then soaked for 10 minutes in 10-percent Clorox bleach.

3. Containers are then rinsed with water, air dried, and stored.

4. Seedling tubes (Ray Leach tubes) that need cleaning are first put into the large black 98-tube holders.

5. Follow the following steps for the Clorox bleach cleaning.

Clorox Bleach Cleaning

1. Load tubes in cleaning rack (black holder).

2. Place eight holders in metal bin outside of soil building.

3. Pour in 7 gallons of Clorox bleach.

4. Fill bin to top with water and cover bin.

5. Allow tubes to soak at least 1 hour (longer will not matter).

6. After soaking, remove holders from bin, place three holders in wire cleaning rack, and close lid.

7. Rinse tubes thoroughly with a high-pressure hose to remove all debris.

8. Check Clorox solution with blotter paper to assure that it is still active (may take up to 15 minutes to turn from blue to pink).

9. Repeat steps 1 through 8 as long as bleach solution is active.

10. Cover bin when not in use (light breaks down bleach).

Note: A 10-percent Clorox bleach solution is used. Measurements must be adapted for different cleaning bins.

INCUBATION CHAMBER

The purpose of the incubation chamber is to give optimum conditions for fungus infection to take place. The optimum temperature and humidity for fusiform rust is 20.5 ^{o}C and 97+ percent humidity at all locations in the chamber.

The temperature is maintained by circulating 20.5 ^{o}C water through the chamber. A refrigerated water bath is used to maintain the water temperature. The water is pumped into a perforated pipe around the inside of the chamber. The water runs down a muslin curtain into a gutter that channels the water to a sump, and the water is then pumped back into the refrigerated water bath.

The humidity is controlled with a humidifier that runs 20 seconds every 2 minutes. The water supply for the humidifier is a water line tapped into the temperature control water pipe of the chamber after it comes out of the refrigerated water bath.

The temperature and humidity are checked with a psychrometer at three different locations on at least three different shelves on each side. The temperature and humidity should be checked each time the shelves are moved or adjusted. The dry and wet temperature should be between 69 and 71 ^{o}F.

The chamber is built out of an 8- by 8- by 8-foot walk-in cooler with 5 shelves (23 by 72 inches) on each side. The shelves are normally set at 18 inches apart, but can be adjusted on 6-inch intervals. The chamber will hold 210 metal holders (10 by 7 inches).

COMMERCIAL COOLER

The commercial cooler located in the soil building is used to stratify and store northern red oak acorns and some pine seed. The cooler should be set to maintain a temperature as close to 33 ^{o}F as possible, but care must be taken to keep the cooler from freezing. This means the thermostat should be set around 35 to 37 ^{o}F.

RSC LABORATORY

The laboratory temperature must be maintained below 81 ^{o}F.

A freezer is needed for long-term storage of seeds.

An incubator or refrigerator is needed for long-term storage of aeciospores. One is also needed for stratification of seed and short-term storage; one for basidiospore processing, storage, and preparation of pine inoculum. All incubators or refrigerators must maintain 5 ^{o}C $\pm$ 1^{o}.

A Coulter electronic counter is used to make counts for the pine inoculum. This unit should be plugged into an isolated electrical circuit to reduce electrical interference in the counts.

Natural gas and air supplies are needed for the natural gas-air torch used to seal ampoules of aeciospores. The air supply is produced by a pressure/ vacuum pump.

GREENHOUSE LIGHTING FOR PHOTOPERIOD CONTROL

The photoperiod is extended with the use of Gro-Lux fluorescent lamps in the glass greenhouse, germination bed, and plastic house 1. Plastic house 2 uses incandescent reflector lamps.

Photoperiod Extension Specifications

1. Oak plants (Bay 1, glass greenhouse) receive 40 foot-candles for 14 hr/day, annually. Lights are controlled by a photocell, which is controlled by a timer. The timer is located in the headhouse and is set to come on at 6 a.m. and go off at 10 p.m. The photocell, located in Bay 1 above the light switches, is set with a light meter by measuring the amount of natural light at the top of oak pots at different locations on the benches. In order to set the photocell there must be no more than 40 foot-candles at the location with the least amount of natural light. Cover the electric eye with the dark slide and/or dark tape until the lights come on. There is a time delay in the photocell; allow 3 minutes between changes in the electric-eye covering for the lights to come on.

2. Germination bed (Bay 2, glass greenhouse) receives 100 foot-candles for 16 hr/day, annually. Lights are controlled by a photocell, which is controlled by a timer. The timer is located in the headhouse and is set to come on at 4:30 a.m. and go off at 8:30 p.m. Measure the amount of natural light at bed level at different locations in the bed. There must be no more than 100 foot-candles at the location with the least amount of natural light in order to set the photocell. Cover the electric eye with the dark slide and/or dark tape until the lights come on. There is a time delay in the photocell; allow 3 minutes between changes in the electric-eye covering for the lights to come on.

3. Pine plants (all greenhouses) receive 30 foot-candles (as measured at tube top) for 12 hr/day, annually. Lights are controlled by timers. Lights are not required from April 15 through September 1 in either plastic house, but are continued in the glass greenhouse. The timers, located in the headhouse and in right-front corner of each plastic greenhouse, are set to come on at 6 a.m. and go off at 8 a.m. or to come on at 6 p.m. and go off at 8 p.m.

GENERAL GREENHOUSE HEATING AND COOLING SPECIFICATIONS

Optimum year-around temperatures are 75 to 85 ^{o}F in the glass greenhouse and 60 to 75 ^{o}F in the plastic greenhouses.

Due to wide variations in day and night temperatures outdoors in the spring and fall, it is nearly impossible to set both heating and cooling system thermostats for any length of time. Therefore, whenever necessary, each system should be adjusted to meet the day's conditions. The cooling systems in Bay 1 of the glass greenhouse should be turned on any time outside humidity drops below 45 percent to maintain high humidity for basidiospore production.

Glass Greenhouse Heating

In winter, temperatures near 75 ^{o}F should be maintained in all bays of the glass greenhouse.

From April to mid-June, heating thermostats should be gradually decreased to maintain a temperature near 70 ^{o}F.

From September to October or mid-November, heating thermostats should be gradually increased to maintain a temperature near 75 ^{o}F.

In winter, if outside temperatures drop below 15 °F, then Bay 2 temperature should be maintained near 85 °F to assure continued even germination of pine seeds in germination bed.

The graduations on the thermostats may not be accurate with the temperature on the benches; therefore, set the thermostats to give the desired temperature at the benches.

In winter, the exhaust fans should be set to turn on about 13 °F above the desired temperature. The vents should be set to open about 17 °F above the desired temperature.

Plastic Greenhouse Heating

In winter, temperatures 60 to 70 °F should be maintained in both plastic greenhouses.

From April to June, heating thermostats should be gradually decreased to maintain a temperature near 60 °F.

From September to October, heating thermostats should be gradually increased to maintain a temperature near 60 °F. The graduations on the thermostats may not be accurate with the temperature on the benches; therefore, set the thermostats to give the desired temperature at the benches.

In winter, the exhaust fans should be set to turn on at 25 °F above the desired temperature. The shutter thermostat should be set to open at 30 °F above the desired temperature.

The fan jet should always be set to come on with the heaters.

In plastic greenhouse 2, the fan jet has a two-way switch; in one position it operates with the heating thermostat and in the other position it operates with the shutter thermostat.

Greenhouse Cooling

Between late April and late September, the evaporation cooling systems, cooling systems covers, exhaust fan, vents, and shutters should be adjusted to keep the greenhouse temperatures from exceeding 85 °F.

In early spring and late fall, the temperature can be lowered by opening the covers which close off the cooling systems and by turning on the exhaust fans.

In mid-May (after the last hard freeze), Kool-Ray shading should be painted onto the glass greenhouse. Bay 1 should have an extra heavy layer to provide extra cooling for the oak plants.

The graduations on the thermostats may not be accurate with the temperature on the benches; therefore, set the thermostats to give the desired temperature at the benches.

The exhaust fans should be set to come on at 70 °F and the evaporation cooling system to come on at 75 °F at the benches.

Vents and shutters in the glass greenhouse and plastic greenhouses should be set to open at 85 °F at the benches.

FAN JET OPERATION

The purpose of the fan jet is to circulate air inside the plastic greenhouses.

Plastic Greenhouse 1 - Winter Settings

Note: Because calibration of different thermostats may vary, their dial settings may differ from the actual temperature desired. To check differences between fan jet and heater thermostats, turn each slowly until the controlled unit comes on; note the difference in settings of the thermostats. The desired temperature should always be measured with a hygrothermograph at bench top level.

1. Set heater thermostat to desired setting.
2. Set fan jet thermostat approximately 1 degree higher than heater thermostat.
3. Set shutter thermostat at 95 °F.

Plastic Greenhouse 1 - Summer Settings

Note: Calibrate thermostats as stated before.

1. Set fan jet thermostat to come on at same time as shutter opens.
2. Set heater thermostat to 40 °F.

Insecticide Settings

1. Set fan jet thermostat to keep fan jet on (highest setting).
2. Set shutter thermostat to keep shutter closed (highest setting).

Plastic Greenhouse 2 - Winter Settings

1. Set heater thermostat to desired setting (after calibration as noted before).
2. Fan jet switch should be in the down position.
3. Set shutter thermostat to open at 95 °F.
4. To circulate air to dry trees, set shutter to open at 30 °F and place fan jet switch in the up position. Shutter is to be turned off with the switch at the shutter.

Plastic Greenhouse 2 - Summer Settings

1. Set shutter thermostat to open at 90 °F.
2. Fan jet switch should be in the up position.
3. Set heater thermostat to 40 °F.

Insecticide Settings

1. Turn off heaters with switches near the heaters.
2. Set heater thermostat to highest setting.
3. Fan jet switch should be in the down position.
4. Set shutter thermostat to highest setting.

Trade names are used in this publication solely to provide specific information. Mention of a trade name does not constitute a warranty of the product by the U.S. Department of Agriculture or an endorsement by the Department over other products not mentioned.

Pesticides used improperly can be injurious to man, animals, and plants. Follow the directions and heed all precautions on the labels.

Store pesticides in original containers under lock and key--out of the reach of children and animals--and away from food and feed.

Apply pesticides so that they do not endanger humans, livestock, crops, beneficial insects, fish, and wildlife. Do not apply pesticides when there is danger of drift, when honey bees or other pollinating insects are visiting plants, or in ways that may contaminate water or leave illegal residues.

Avoid prolonged inhalation of pesticide sprays or dusts; wear protective clothing and equipment if specified on the container.

If your hands become contaminated with a pesticide, do not eat or drink until you have washed them. In case a pesticide is swallowed or gets in the eyes, follow the first aid treatment given on the label, and get prompt medical attention. If a pesticide is spilled on your skin or clothing, remove clothing immediately and wash skin thoroughly.

Do not clean spray equipment or dump excess spray material near ponds, streams, or wells. Because it is difficult to remove all traces of herbicides from equipment, do not use the same equipment for insecticides or fungicides that you use for herbicides.

Dispose of empty pesticide containers promptly. Have them buried at a sanitary land-fill dump, or crush and bury them in a level, isolated place.

Note: Some States have restrictions on the use of certain pesticides. Check your State and local regulations. Also, because registrations of pesticides are under constant review by the U.S. Environmental Protection Agency, consult your State forestry agency, county agricultural agent or State extension specialist to be sure the intended use is still registered.